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LONGMANS, GREEN AND CO.,

THE PHYSIOLOGY

OF

PROTEIN METABOLISM

BY

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TO

D. N. P.

" Entia non sunt multiplicanda præter necessitatem."

GENERAL PREFACE.

THE subject of Physiological Chemistry, or Biochemistry, is enlarging its borders to such an extent at the present time, that no single text-book upon the subject, without being cumbrous, can adequately deal with it as a whole, so as to give both a general and a detailed account of its present position. It is, moreover, difficult, in the case of the larger text-books, to keep abreast of so rapidly growing a science by means of new editions, and such volumes are therefore issued when much of their contents has become obsolete.

For this reason, an attempt is being made to place this branch of science in a more accessible position by issuing a series of monographs upon the various chapters of the subject, each independent of and yet dependent upon the others, so that from time to time, as new material and the demand therefor necessitate, a new edition of each monograph can be issued without re-issuing the whole series. In this way, both the expenses of publication and the expense to the purchaser will be diminished, and by a moderate outlay it will be possible to obtain a full account of any particular subject as nearly current as possible.

The editors of these monographs have kept two objects in view: firstly, that each author should be himself working at the subject with which he deals; and, secondly, that a *Bibliography*, as complete as possible, should be included, in order to avoid cross references, which are apt to be wrongly cited, and in order that each monograph may yield full and independent information of the work which has been done upon the subject.

It has been decided as a general scheme that the volumes first issued shall deal with the pure chemistry of physiological products and with certain general aspects of the subject. Subsequent monographs will be devoted to such questions as the chemistry of special tissues and particular aspects of metabolism. So the series, if continued, will proceed from physiological chemistry to what may be now more properly termed chemical physiology. This will depend upon the success which the first series achieves, and upon the divisions of the subject which may be of interest at the time.

R. H. A. P.
F. G. H.

PREFACE TO NEW EDITION.

In spite of the fact that this small volume is, from the very plenitude of material at one's disposal, compressed in undue measure, an attempt has been made to render this intricate problem of physiology exoteric.

The work started by Liebig, Voit, Pflüger, Zuntz, Rubner and their pupils is now being steadily added to, mainly in the last few years, by workers in the United States, above all by Lusk, van Slyke, Folin, Osborne, Mendel, and many others.

No attempt has been made to discuss in detail the part played by the accessory food substances on the course of metabolism as a special monograph dealing with this phase of the work is projected.

In addition to bringing the material up to date, many sections have been rewritten. A final chapter summarising the influence of the non-nitrogenous food-stuffs on the metabolism of protein has been added.

It is a pleasure to express my indebtedness to my wife for much helpful criticism.

E. P. C.

November, 1920.

PREFACE TO FIRST EDITION.

More work has perhaps been done upon the digestion and assimilation of proteins than upon any of the other branches of metabolism. A monograph on Protein Metabolism would therefore be the first of a series, and it is to be hoped that volumes dealing with the other particular aspects of metabolism will soon be forthcoming which will furnish us with a complete survey of the present position of this portion of chemical physiology.

The present monograph does not pretend to cover the whole literature of protein metabolism; it consists rather of the discussion of the more important results published during the last decade and their bearing upon the work of the earlier investigators. The majority of recent writers have devoted their attention to the study of the metabolism of particular constituents of the protein molecule; an attempt has been made in this monograph to avoid laying undue stress on the fate of these since it is felt that a truer picture of the real course of protein metabolism can thus be drawn.

It is a pleasant duty to express my indebtedness to Miss G. D. Bostock, M.B., for her valuable assistance in the revision both of the manuscript and the proofs.

E. P. C.

1912.

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INTRODUCTION.

FACED with the question as to the nature of the processes which lead to the building up of the material commonly known as "tissue protoplasm," we are at the very outset hampered and confined in our quest for information by the imperfect knowledge which exists as to the very nature of the material formed. Can we with right assume that such a substance actually exists as a constant chemical entity, a substance immutable in form but variable in quantity? Does it wax and wane as does a crowd, the units constituting the whole, inconstant in number but identical in nature, or is it a material unstable alike in form and amount? As Sir Michael Foster wrote in 1885: "He (the biologist) may speak of protoplasm as a complex substance but he must strive to realize that what he means by that is a complex whirl, an intricate dance of which what he calls chemical composition, histological structure and gross configuration are, so to speak, the figures; to him the renewal of protoplasm is but the continuance of the dance, its functions and actions the transference of figures".

The term metabolism embraces both phases of cellular activity, the change or transformation of energy, in the main a catabolic phenomenon, in which the special nature of the material broken down probably plays a secondary rôle, and the change of material where both anabolic and catabolic phenomena are involved, and where the nature of the material supplied plays an all important part. The whole question of the relation of the change of material to the change of energy, although it is one of paramount importance, has only been referred to incidentally in the present monograph. Both sides of the problem are comprehensively and admirably dealt with in Lusk's [1917] unique and indispensable book. In spite of the evidence put forward by Rubner and others, I am strongly of the opinion in the first place, that the two exchanges can be considered separately, although it is admitted that the picture may be incomplete, and in the second place, I would maintain that there are phases of the metabolism of protein with which the dynamic aspect of

the question has but little to do. No doubt the living organism may be considered as a transformer of energy, but it is a transformer not constructed of stable material, a transformer which besides utilizing the incoming food-stuffs for the generation of energy also uses them for the making good of wear and tear and for increase in bulk.

We cannot, therefore, ignore the fact that just as there is a minimum level for the intake of the various food materials for the reparation of wear and tear, there is a minimum energy level. Numerous investigators have shown beyond cavil that physiologists are justified in speaking of the basal or standard metabolism of an organism. Are we justified in thinking of the cellular activity in terms of a slow combustion device and that on this slow energy transformer there can be superimposed a definite and specific generation of excess energy from extra fuel consumed, a generation of energy which both in kind and degree is peculiar to the particular food-stuff utilized? To take a concrete simile, are we to look on these cellular activities as being more or less equivalent to a well-controlled gas fire, oil is thrown on to it, there is a great and sudden increase in the output of heat but no increase, of course, in the original flame. Must the materials utilized by the cell be first converted into an integral part of the cell or can they be utilized before incorporation? In the crude simile just used it is easy to demonstrate that the molecular form of the added material plays a very important rôle; thus a handful of finely powdered carbon in the form of coal dust would be most effective as a source of energy, whereas a handful of diamond dust would be useless. It is now being appreciated, mainly from the work of Dakin and his collaborators, that the molecular structure of the food materials plays an equally important rôle in the organism. Still more recently it has been shown by Hewitt and Pryde that in all probability stereochemical changes play an important part in the absorption, if not in the metabolism, of such relatively simple compounds as glucose. It may well be that these molecular structural changes will prove to have as profound an influence on the finer metabolic phenomena as deficiencies in amino acid content have, for example, on the grosser.

The fact must not be overlooked that there has been some confusion of ideas in connexion with the use of the term protein metabolism. The term protein metabolism must not, as is often done, be used simply as a synonym for the metabolism of protoplasm or the total metabolic activity of the living cell. The metabolism of protein is insolubly bound up with the metabolism of other products. Protein

metabolism, if it ever takes place alone, can never be anything but a mere episode in cellular activity.

Implicitly, or explicitly, it has been the custom to regard the catabolic phase of cellular activity as the preponderating phase, and yet workers like Loew [1915] maintain that the change of a labile to a stable modification is easier than the change in the opposite direction. This distortion of the actual facts of the case is mainly the result of the elaborate methods at our disposal for the detection of the products of catabolism. It is self-evident that, under normal conditions, when the organism is in a state of equilibrium, the two forms of activity, the anabolic and catabolic, will be approximately equal.

Still, abstraction or no, the method of disentangling one substance by differentiating its metabolism, be it only true in part, is the only satisfactory method of dealing with metabolism in detail. It does not mean, however, that the metabolism of any one product is of greater primary importance than any other. Protein, however, holds a peculiar position as it is the source of nitrogen for the organism. All life processes are undoubtedly bound up with protein; it plays a conspicuous part in the cellular drama. One of the most interesting points about this protein compound is that it exists in so many different forms; it is so frequently specific in its molecular structure, and some of these specificities are almost strictly confined to single organs. It is the disintegration of the specific protein to its constituents which are, for the most part, non-specific, which would seem to be the characteristic function of digestion; the breakdown of the colloidal, non-dialyzable, whole protein to the dialyzable simple peptides and amino acids.

The problem of metabolism in general and of protein metabolism in particular is undoubtedly one of the most complex and obscure in physiology because the causes which bring about the changes are practically unknown. Carl Voit, the master and founder of modern metabolic research, even in 1902, after forty years of strenuous work in this field, could say no more than that "the unknown causes of metabolism are found in the cells of the organism. The mass of these cells and their power to decompose materials determine the metabolism." Strive as we may our insight into this intricate problem of the nature and changes of living matter is but faulty. We see through a glass darkly.

CHAPTER I.

DIGESTION AND ABSORPTION OF PROTEIN.

General Methods of Investigation.

THE elucidation of the problems of protein metabolism has been attempted in various ways. The usual and most commonly employed method is that of studying the output of nitrogen in the urine, particularly the variations which occur in the partition of the different nitrogenous constituents. Since the careful and painstaking work of Folin [1905, 1, 2] and the publication of his analyses of the composition of the normal urine upon different diets the conclusions have assumed an accuracy and weight which previously were lacking.[1] The fact, however, must not be lost sight of that the mere study of the nitrogen balance does not give any real index of the changes which take place in the tissues. The nitrogen excreted is surplus nitrogen arising from both endogenous and exogenous sources.

The variations in the output of nitrogen have been investigated under different conditions, thus (1) during complete starvation, (2) after definite alterations in the amount of one or more constituents present in the diet, as, for example, the production of nitrogen hunger on a diet which supplies the requisite number of calories, but which is lacking in protein or other substances containing nitrogen, (3) a diet which, although rich in nitrogen, lacks either carbohydrate or fat, (4) a diet in which the normal protein of the food is replaced by some nitrogen-containing substitute, i.e. by the addition of cleavage products of protein formed either by the action of a ferment, or an acid.

Observations have also been made when the protein has been introduced parenterally by intravenous, intraperitoneal or subcutaneous injection. The influence of various extraneous factors like work has also been investigated.

The problem has again been attacked in another fashion by means of perfusion of different organs and tissues with substances of known

[1] An excretion of nitrogen by way of the skin does take place in water soluble form amounting, as Benedict [1915] has shown, to as much as 0·1 grm. per day. The output can be considerably increased by muscle work.

constitution or which give a well-defined reaction. Such experiments must be interpreted with the utmost caution. In so far as they are carried out on isolated organs they are non-physiological: the organism does not work with a set of insulated and completely dissociated units. One of the most outstanding facts of modern research is the intimate correlation which has been shown to exist between the different organs and tissues of the body. The same thing has also been definitely shown to hold for metabolic activity. Of course it is freely admitted that in many instances results obtained by perfusion methods have been fully substantiated by the more physiological feeding methods.

The subject has also been investigated by studying the fate of particular substances when the organism is in a pathological condition. The products excreted in the urine in cases of alkaptonuria and cystinuria,[1] or in such a disease as acute yellow atrophy of the liver, have been examined, both after normal feeding and after the addition of different amino acids, etc., to the food. The course of metabolism has also been studied in pathological conditions artificially produced, as, for example, after phosphorus poisoning.

The discussion of the question of metabolism is opened with a short preliminary résumé of the present position as regards the course of gastro-intestinal digestion. This is inserted, as it is impossible to obtain a proper understanding of the subsequent fate of the protein without some knowledge of the course and extent of digestion in the gastro-intestinal tract.

Gastric Digestion.

Actual digestion of protein does not commence until the material has reached the stomach, where it is subjected to the action of gastric juice—pepsin and hydrochloric acid. A great deal of work has been done on the extent and degree of digestion which takes place here. It may be assumed that under normal conditions the digestion only proceeds as far as the peptone stage, but that all protein does not of necessity reach this stage of degradation. Lavroff [1899], Langstein [1902], and others, however, have clearly demonstrated that if the gastric digestion be allowed to go on long enough, *in vitro* at least, the breakdown of protein can be carried on to the formation of abiuret products, i.e. products of digestion which no longer give the biuret

[1] In the present monograph this side of the question will not be specifically considered. For further information Dr. Garrod's valuable book on "Inborn Errors of Metabolism" may be consulted.

reaction. This degree of digestion need not be considered here, for even under the most favourable conditions *in vitro* very many weeks are required.

It would seem, from many different observations, that proteoses form by far the largest part of the products of peptic digestion. The amount of proteose formed depends to some extent on the nature of the protein which has undergone digestion. The following table from an article by London[1] shows this very clearly:—

Nature of Protein Fed.	Percentage of Proteose Formed.
Egg albumin .	72·5
Gliadin . .	67·7
Edestin . .	60·3
Caseinogen .	59·1
Gelatin . .	50·6
Serum albumin .	46·1

According to Tobler [1905], on the other hand, from 50 per cent. to 57 per cent. of the digested product reaches the intestine in the form of peptone, some 11 per cent. to 14 per cent. in the form of proteoses, and from 30 per cent. to 34 per cent. in the form of soluble or insoluble protein. Zunz [1907] concludes that three-fifths of the nitrogen of the products of protein digestion in the stomach enters the duodenum in a very simple form (mainly peptones) and about two-fifths in the form of proteoses. He is therefore inclined to agree with the work of Tobler. Zunz further shows that the condition in which the food is consumed plays a part in the degree of digestion reached. Thus when a dog is fed with cooked meat there is more proteose present in the stomach contents than when the same food (horse flesh or beef) is given raw. He also states that, in certain experiments which he carried out in dogs, where, previous to the digestion experiment, the ducts of the pancreas were ligatured, digestion in the stomach was more complete than when the pancreas was acting freely. He concludes that there may be at times an increased compensatory digestion in the stomach.

The degree of gastric digestion would not seem to be a matter of very great moment, as under normal conditions these changes in the stomach are only preparatory to the action of the pancreatic and intestinal juices. That this preparatory action is of importance, however, is shown by the *in vitro* experiments of Fischer and Abder-

[1] London, "Handb. d. Biochemie," Oppenheimer, III, p. 77.

halden [1903]; they showed that tryptic digestion took place much more rapidly and completely when the protein had been previously subjected to the action of pepsin and hydrochloric acid. They found that, if caseinogen were first digested with an artificial gastric juice before digestion with trypsin, they could isolate proline and phenylalanine, whereas if pancreatic digestion of caseinogen were carried out alone, these amino acids were not found in the free state but only in the peptide form. Even the peptide compound was not completely decomposed by the double digestion. Acid hydrolysis, on the other hand, breaks up this peptide completely, both of the amino acids being liberated. Fraenkel [1916] and Abderhalden and Pettibone [1912] have fully confirmed the observation that previous digestion with pepsin leads to very thorough disruption of the protein molecule on subsequent digestion with trypsin and erepsin. Fischer and Abderhalden [1903] and Abderhalden, London and Voegtlin [1907] showed in a long series of experiments that a large number of the polypeptides were resistant to the action of the gastric juice, although they were rapidly broken down by pancreatic juice. Andersen [1915] has also dealt with this problem in a long and interesting series of experiments carried out both *in vitro* and *in vivo*. His work was confirmatory. Oppenheimer and Aron again [1904] found that serum, which is normally resistant to the action of trypsin, could be digested by this enzyme if it had been previously subjected to the action of pepsin, and Dakin and Dudley [1913] have shown that casein, normally very readily digested, after racemization is resistant not only to the action of pepsin but also to trypsin and erepsin.

Gastric Absorption.

There has been much discussion as to whether absorption takes place in the stomach or not. London and his school deny that absorption ever takes place, whereas Tobler [1905] states that after a protein meal he has observed the disappearance of from 22 to 30 per cent. of this material from the stomach. Salaskin [1907] believes that Tobler is right in his contention that absorption of protein can and does take place in the stomach, but he does not bring forward any convincing evidence or experiment in support of his belief. In this contention they are supported by Folin and Lyman [1912, 1, 2]. If this absorption be as great as Tobler makes out, it is extraordinary that it has escaped the observation of so many of the competent workers in this field. Abderhalden, Prym and London [1907] have, for example, shown that even if amino acids be given *per os* they

leave the stomach practically completely through the pylorus, absorption first taking place in the duodenum.

The present general conclusion would seem to be that if any absorption of protein digestion products takes place from the stomach under normal conditions, it must be a small one.

Intestinal Digestion.

The main digestion of protein takes place in the small intestine, most actively at the upper end, by means of the trypsin of the pancreatic juice and the erepsin of the intestinal wall. Trypsin acts on all forms of protein which have been passed on from the stomach and reduces them to simpler products. The question as to the extent of this splitting has been much discussed. Formerly it was believed that proteoses and peptone were the end products, but it is now generally held that the main digestion proceeds to the formation of abiuret products in the form of peptides and the comparatively simple monoamino and diamino acids. (Abderhalden, Baumann and London [1907], Kutscher and Seemann [1901], Cohnheim [1906].) The difficulty has been to prove that the digestion proceeds to this extent, as (1) the disintegration of protein does not take place suddenly, in an explosive fashion, but proceeds gradually, more like erosion, and (2) along with this slow decomposition there is a steady absorption of the simple products as they are formed. By the utilization of the polyfistular method, elaborated by London [1907], [1908], [1909], evidence of the thoroughness of the decomposition has been obtained. Abderhalden, London and Oppler [1908], for example, traced the appearance of tyrosine and glutamic acid after feeding with gliadin. They found in the duodenum 0·75 gm. of tyrosine and 2·5 gm. of glutamic acid, in the jejunum 1·1 gm. of tyrosine and 20·9 gm. of glutamic acid, and in the ileum a mere trace of tyrosine and 33 gm. of glutamic acid. Very similar results were obtained, by the same method, after feeding with caseinogen and with meat. This work was repeated and confirmed later by Abderhalden, London and Reemlin [1908], and Abderhalden [1912, 3]. Underhill [1911] has shown that the short-circuiting of even large sections of the intestinal canal has but little influence on the utilization of food. Not only then has it been proved that the digestion is gradual, but further, that the rate of digestion is greater than that which takes place *in vitro*. But although gradual it must not be thought of as a comparatively slow process. Janney [1915, 2] found that the time required by the organism to effect digestion of the complex protein molecule into

amino acids and to metabolize them further is but little longer than that required for the absorption and elimination of dextrose. Van Slyke [1917], too, showed that absorption began, as indicated by blood analyses, immediately protein material passed the pylorus.

Abderhalden and Gigon [1907] have demonstrated that the digestive ferments can combine with the amino acids formed in the course of digestion and in this way become inactivated. This inactivation is liable to occur during *invitro* experiments, where the amino acids accumulate in the digestive fluid, but in the case of the ntestine, as absorption is constantly taking place and as fresh supplies of ferment are always available, there is but little slowing of the rate of digestion. Abderhalden and Rona [1906, 1] have further shown that there is another difference between the actions of artificial juices, both gastric and pancreatic, and the real juices in *in vitro* experiments. They found that pepsin powder can rapidly digest caseinogen giving rise to various amino acids and tryptophan, whereas the real gastric juice, when tested under like conditions, cannot do so. They also found that "pancreatin" will break down many polypeptides which are resistant to the action of natural pancreatic juice. This is proof, if proof be required, that there is considerable danger in drawing far-reaching conclusions, as regards intravital action, from mere test tube experiments, important and useful though the latter be.

Intestinal Absorption.

It was not until the experiments of Salvioli, Hofmeister and others were carried out that a new explanation of the mode in which the proteins were absorbed from the intestine was sought. Salvioli [1880] and Hofmeister [1882] almost contemporaneously made the discovery that, if peptone were left in contact with the living intestinal wall, it disappeared or at any rate was so altered that it no longer gave the reactions by which it was characterized. Hofmeister concluded that the peptone on absorption was taken up by the leucocytes of the intestinal wall and by means of these was converted into protein and at once conveyed to the tissues [1885]. Heidenhain [1888] repudiated this hypothesis, but both he and Shore [1890] inclined to the view that the peptone was converted into protein, and that in this change the epithelial tissues of the intestine probably played an important part. In addition to this Hofmeister [1881] and others have shown that if peptone were injected into the blood it was wholly or in greater part excreted from the body as waste material, and further that no trace of peptone was ever found in the tissues, blood or lymph of

animals even at the height of digestion. Neumeister [1897] found in the intestinal mucosa two decomposition products of protein, leucine and tyrosine, which suggested at least that further breakdown had taken place. The presence of these amino acids, however, did not necessarily exclude the possibility of a subsequent synthesis—the condition might be analogous to the splitting of fats into fatty acids and glycerol which precedes the fat synthesis in the intestinal mucous membrane. Cohnheim [1901] attempted to isolate the protein which he believed was synthetized in the intestinal wall. He found that an increase of protein, i.e. a regeneration, never took place, but that the peptone was invariably broken down to simpler decomposition products; in other words that the characteristic peptone reaction disappeared not because protein had been synthetized but because abiuret products were formed. He further discovered the ferment which brought about this decomposition, and to it he gave the name erepsin. Kutscher and Seemann [1901] on investigating the fate of protein in a dog with a fistula in the middle of its small intestine found that the protein—flesh—was reduced to amino acids of which leucine, tyrosine, lysine and arginine could be isolated, but neither proteose nor peptone was detected. This observation, that leucine and tyrosine could be isolated from the normal intestinal contents, was by no means new, as Kölliker and Müller as long ago as 1856 had discovered leucine and tyrosine in the intestinal contents, although of course they were unable to assign an explanation to their presence. They concluded that they were either absorbed or broken down further as they were unable to find them in the fæces.

Kühne [1867] also detected leucine and tyrosine in the material collected from an isolated loop of intestine, and he rightly described them as being products arising from the breakdown of protein, but thought that they were rather bye-products than normal digestion products on the way to absorption. Schmidt-Mulheim [1879], who repeated Kühne's work, came to the conclusion that although such a breakdown took place it was quite unimportant. Sheridan Lea [1890] came to similar conclusions as Kölliker and Müller. Macfadyen, Nencki and Sieber [1902], who investigated the case of a woman with a fistula at the lower end of the small intestine, found that the intestinal contents contained soluble proteins and peptones, but no leucine or tyrosine.

Salkowski and Leube [1880], on the other hand, put forward the suggestion that the leucine might be considered as a product which after absorption could be used for rebuilding purposes, and which

therefore might be regarded as a stage towards protein regeneration, analogous to the conditions ruling in plant physiology where it had been demonstrated that the decomposition products of protein, asparagine, leucine and tyrosine could be regenerated into protein when carbohydrate was also present. Kutscher and Seemann [1901], as the result of their experiments, stated that they considered the above hypothesis very plausible, and concluded that the appearance of leucine and tyrosine which they found, was the normal condition, and that these crystalline substances must be looked on as material, which, after absorption, would be utilized for the formation of tissue protein. They were never able, however, to detect amino acids in the blood even at the height of digestion. It is interesting to note that Bunge in his textbook argued on teleological grounds against the conversion of any considerable amount of protein into amino acids. He held that if any such conversion took place it must be small, as the dissipation of chemical energy firstly in the decomposition and secondly in the necessary building up processes would be considerable and quite contrary to nature. These teleological arguments of Bunge can now be shown to be false. The loss of energy in the conversion of protein into digestion products is remarkably small, as Rubner's calorimetric estimation of Loewi's digestion products proved. Even in plants, in the process of utilization of the stored protein, the formation of crystalline decomposition products must take place before resynthesis is possible.

Absorption of Undigested Protein from the Intestine.

The question as to whether the undigested native protein can be absorbed must also be considered. Magnus Levy has suggested that although the greater part of the protein is broken down to simple nuclei, which are simply burnt up without playing any part in the tissue metabolism, the body may absorb from the intestine a sufficient amount of unaltered protein for purposes of repair of tissue. That the body can absorb protein in the natural form has long been known. Voit and Bauer [1869] showed that the absorption of undigested proteins such as serum and uncoagulated egg albumin could take place, and their results have been extended and amplified by Heidenhain [1894], Friedlander [1896], Waymouth Reid [1900] and others. It is not maintained, however, that this is the usual way in which most of the absorption takes place. Ascoli and Vigano [1903], using the biological precipitin test, have stated that they were able to demonstrate that part of the protein was taken up unchanged.

Abderhalden, Funk, London [1907], under much better conditions than Ascoli, and also using the biological method, were quite unable to obtain any reaction. In all these experiments the protein was introduced into the intestine in excessive amount. This absorption, such as it is, would appear to be dependent to some extent on an increased permeability of the intestinal wall, such as is found in the young. It is also dependent to a certain extent on the presence of water or salt solution. Friedlander [1896], for instance, has shown that, if all the water or salt solution be absorbed, the absorption of the proteins to all intents and purposes comes to a standstill. Naturally doubts have been thrown on this form of absorption. It has been suggested that the intestinal digestion had not really been suspended, although the intestine previous to the introduction of the protein solution had been thoroughly washed out, i.e. that a certain amount of pancreatic juice had been left which brought about a solution of the protein, and thus a natural absorption. This objection is not valid, as the protein was rapidly absorbed, and further the amount of enzyme which could have been present must have been small and its activity on native protein slight. For example, the figures quoted both by Heidenhain [1894] and by Waymouth Reid [1900] show that the intestine can deal with large amounts of protein in a very short period of time.

Omi [1909] has made a curious observation in connexion with the absorption of native protein. He found that dog serum is readily absorbed from the dog's intestine, but that if horse or ox serum be employed absorption only takes place with difficulty and in small amount. If, however, the "ox" serum be put into the intestinal loop along with an equal amount of ox pancreatic extract, absorption is quite marked. The greatest absorption of all, however, followed the placing of a mixture of dog serum and dog pancreatic extract in the loop.

Fate of Parenterally Introduced Protein.

Even supposing the protein can be taken up to any extent in an unaltered condition, the question naturally arises, can the body deal with native protein circulating in the body fluids—in other words, is parenterally introduced protein of value to the organism? The work of many investigators, as Zunz and Mering [1883] and Neumeister [1897], among the older workers, has shown that utilization of native protein introduced parenterally does take place. Sollmann and Brown [1901] have demonstrated clearly that under favourable conditions egg albumin injected intravenously can be well utilized, in

many instances only a mere trace of protein appearing in the urine. On the other hand, Gürber and Hallauer [1904] hold that the non-appearance of the protein in the urine is no evidence of its utilization in the tissues. In their experiments they injected a solution of caseinogen intravenously and found that part of this material reappeared in the urine, but that part was also excreted into the intestine by way of the bile. This excretion into the intestine and the subsequent digestion and absorption might of course account for the positive results which have been obtained. Friedemann and Isaac [1905] showed that, if subcutaneous injections of egg white or of serum were given to dogs and goats, the material was for the most part excreted in the urine in a non-coagulable form; in other words, although it was not utilized it had been attacked during its stay in the tissues. Examination of the blood four hours after the injection of 400 c.c. egg albumin showed the presence of a non-coagulable biuret-giving body (? proteose), but neither polypeptides nor monoamino nor diamino acids were found. In goats, in a condition of starvation, they found that there was a retention of the injected nitrogenous material before immunity was induced (by the repeated injections of protein), whereas after the induction of immunity the result of further injections of the protein was to bring about a marked rise in the output of nitrogen in the form of urea, frequently even exceeding in amount that of the nitrogen injected. They also carried out a few experiments in which they injected a dog fed on a carbohydrate diet (potatoes) and observed a well-marked retention of nitrogen without any precipitin formation. They continued their investigations [1907] and found that the condition of the state of nutrition of the animal played a very important part in this utilization of protein. It was absolutely immaterial whether the protein injected into the starving animal was the animal's own serum, or foreign serum, or egg albumin; there was in each case an actual increase in the breakdown of tissue protein as the rise in the nitrogen excretion exceeded the amount of nitrogen injected. When, however, the animal was on a carbohydrate diet no increase in nitrogen excretion was found to follow the injection, indeed a retention of nitrogen took place. This retention was so great that they were able to keep an animal in nitrogenous equilibrium by protein administered parenterally. That the carbohydrate played an essential part is shown by the fact that if the animal be put on a carbohydrate free diet and injected there is an increase in the output of nitrogen similar to that which occurs in starvation. They could not prove, however, that any addition of actual body substance

occurred. They came to the general conclusion that parenterally introduced protein could be broken down and utilized by the body, but that the precipitin reaction was of no value for these investigations, since, although any excess of the injected protein might be eliminated in a day or two, the precipitin reaction persisted for a much longer period. Naturally it has been suggested that the Ehrlich theory would be applicable to the utilization of protein material, but Noda [1909] was unable to obtain any positive evidence which would support the view that "protein amboceptors" existed. Heilner and Schneider [1913] were of the opinion that complement does stand in some close relation to protein metabolism but their evidence was not satisfactory. Heilner [1912] has shown, however, that if foreign blood serum be injected into a normal animal subcutaneously it is all utilized within three days, maximum breakdown taking place on the third day, but if the injection be made when the animal is anaphylactic, instead of an increased there is actually a markedly diminished protein metabolism.

Mendel and Rockwood [1905] have shown that edestin and excelsin, when introduced into the circulation, can apparently be retained in the organism, at any rate they are not eliminated unchanged in the urine; when introduced into the peritoneal cavity they were also found to disappear. Borchardt [1907], like Gürber and Hallauer [1904], found that the urine was not the only channel of excretion for parenterally introduced proteins; the non-appearance in the urine of the substance injected could not therefore be accepted as absolute evidence of utilization. This worker injected intravenously hemielastin and observed that a part of it could be detected in the wall of the small intestine. He concluded, therefore, that this material was either on its way to the intestine for excretion or to undergo certain changes which would render it suitable for utilization by the body, or that it was in process of absorption after excretion by way of the bile. The latter hypothesis was not considered likely, as no trace of hemielastin was discoverable in the liver. Michaelis and Rona [1908, 1] made an attempt to replace part of the nitrogen in the diet (meat, milk, etc.) of an animal in nitrogenous equilibrium by injecting an equivalent amount of caseinogen subcutaneously. They found, as Friedemann and Isaac had previously found, that there was an increase in the output of nitrogen in the urine. The caseinogen was not excreted in the urine as such, and like many previous workers, they concluded that it had been broken down. They suggested, as the result of some of their experiments, that the mammary gland might be looked on as a

channel of excretion for caseinogen. In favour of this view is the fact that the mammary gland has been shown by Engel and Murschhauser [1911] to have a definite excretory function so far as urea is concerned. They found that the administration of urea to lactating women was followed by an increased urea output in the milk. Aaron [1917], on the contrary, found in dogs that about 58 per cent. of the casein nitrogen injected intravenously appeared in the urine apparently in the form of casein. He cited some work of Quaglieriello where, following the injection of muscle protein, there was but a slight excretion of protein in the urine. Michaelis and Rona also found later [1908, 2] that, if horse serum were injected into dogs, the nitrogen equilibrium could be maintained, and they concluded therefore that the tissue cells could to a certain extent take on the specific function of the intestinal mucous membrane. They only observed excretion of protein in the urine after a very large dose of serum. Orenstein [1912] also found that for a limited period (8-12 days) dogs could be fed by subcutaneous injections of foreign blood serum and dextrose but, if continued too long, death resulted presumably from anaphylactic shock. The addition of oil to the injection material merely increased the rate of protein catabolism and brought about the earlier death of the animal. Heilner [1908] has suggested that this utilization of injected protein is brought about by the generation of a special ferment. He found that the injected serum was well utilized (see also p. 19). Whatever the nature of the breakdown may be Somogyi [1911] has shown that the composition of the urine varies with the mode of administration of the protein. Subcutaneous administration of serum protein, for example, led to a much more marked rise in the percentage output of ammonia than when the same protein was given by the mouth. Kjöllerfeldt [1917], who investigated the rate of absorption, from the peritoneal cavity, of milk, casein, peptone, hydrolyzed casein and sundry amino acids, maintained that there was no true absorption of milk and casein but that the other substances were rapidly taken up, 24 per cent. of the peptone, 40 per cent. of the hydrolyzed casein and 50 to 60 per cent. of the amino acids in the course of an hour.

A series of experiments have also been carried out in which the parenterally administered protein was in the form of digest products. Buglia [1912, 1, 2] found that, provided the injection were slowly made, a large amount may be introduced. He thought, although his evidence is not very good, that the digest products could replace protein. Henriques and Andersen [1913], by a most ingenious method of continuous injection, were able to keep a goat alive for

twenty days by the parenteral administration of a trypsin erepsin digest of goat's flesh containing some 15 per cent. of its nitrogen in peptide form. It was, moreover, no mere question of keeping the animal alive: there was actually a retention of nitrogen, a positive daily balance of about 1·8 gm. for the first eighteen days of the experiment. They obtained a similar result when a digest of calves' flesh was given. Later [1914] they attempted to obtain nitrogen retention, using the same method, with ammonium acetate or urea but without success. Oehme [1914] also obtained evidence of the retention or utilization of nitrogen introduced intravenously. The injected digest products did not, however, prevent the breakdown of tissue protein.

Freund and Popper [1909] carried out a series of interesting experiments in which they examined the blood of animals with and without the intestine cut out of the circulation. They found five minutes after the intravenous injection of a solution of peptone or other product of protein digestion that about 50 per cent. of the injected material could not be recovered, due simply to its distribution throughout the body. Of the other 50 per cent. they found that, if the intestine was in the circulation, only 15 to 20 per cent. was recoverable from the blood after twenty minutes, whereas with the intestine out of circulation practically the whole 50 per cent. was recovered. They demonstrated further that in the first instance about 32 per cent. of the material recovered was so far broken down that it no longer gave a precipitate with tannic acid, whereas when the intestine was absent only some 12 to 18 per cent. was thus changed. They could obtain no direct evidence to show that any part of the injected material was changed into a coagulable form. This work, of course, is strong evidence in favour of the contention that the intestine plays some important part in the preparation even of parenterally introduced protein before it is utilized by the tissues. Körösy [1911], on the other hand, removed practically the whole of the intestinal canal and injected either horse blood serum or vitellin. Mere traces of unchanged protein appeared in the urine. The nitrogen output, too, only showed a slight and irregular rise over that of starvation. Körösy examined the blood of his animals after injection and found that both the water and the protein injected rapidly disappeared from the circulation.

CHAPTER II.

PROTEIN REGENERATION.

HOW and in what form is protein normally conveyed from the intestine to the tissues? It has been shown that it can be absorbed in a natural undigested condition (p. 11), and it has also been shown (p. 12) that protein which reaches the blood stream through other channels than passage through the intestinal mucous membrane can also be utilized.

Are Proteoses or Peptones Found in the Blood?

After the work of Neumeister [1897] it was generally believed that no proteose or peptone could be detected in the blood, but Embden and Knoop [1903], as the result of their experiments on the fate of proteoses and peptones when brought into contact with the intestinal mucous membrane, stated that, if the tests were carefully enough carried out, these substances could be detected in the blood. They held that such absorption must take place, as they claim to have demonstrated that peptone is neither synthetized to a higher product —a coagulable protein—nor broken down to an abiuret product when brought into contact with the intestinal mucous membrane; they could not, however, always demonstrate the presence of this non-coagulable biuret-giving substance in the blood. Langstein [1903] confirmed the observation of Embden and Knoop, but at the same time admitted that the evidence adduced for the presence of proteoses and peptones in the blood was not absolutely convincing. Bergmann and Langstein [1905] maintained, however, that the biuret-giving substance was proteose, and that it must be regarded as a constant constituent. Kraus [1906] also found that small amounts of proteose could be constantly detected in the blood. Recently Abel, Pincoffs and Rouiller [1917] have come to the conclusion, in agreement with Nolf [1907, 1, 2], that proteoses can be absorbed as such from the intestine. They admit that they have been quite unable to detect them in the blood, indeed they were unable to detect them by their most delicate methods even after the addition of known amounts of proteoses, but, as they have

been able to obtain small amounts from the gastro-intestinal mucosa on the one hand and from striated muscle, thyroid and other tissues on the other hand, they have come to the definite conclusion that proteoses are taken up as such (see p. 9).

Schumm [1904], on the other hand, was quite unable, either in health or disease, to detect proteose in the blood. Abderhalden and Oppenheimer [1904] held that, if proteose were present, it was present in amount that could not be detected by the ordinary methods; they therefore maintained that it could not be regarded as a normal constituent of the blood even under the most favourable conditions. For instance, in their experiments three dogs, which had been starved for several days, were given a full meat meal and then killed at the height of digestion and absorption—six to eight hours after the meal. On thorough examination of the blood no trace of a biuret-giving substance could be detected. They put forward the view that the so-called presence of proteose—or at least of the biuret reaction—was due to the imperfect methods by which the blood was coagulated, i.e. that traces of the coagulable protein were left which sufficed to give a definite biuret reaction. Freund [1908] refused to accept this explanation. He maintained that Abderhalden by his technique precipitated not only protein but every trace of proteose. Apart altogether from imperfect coagulation it is possible that the biuret reaction is due to the presence in the blood of non-coagulable proteins or of proteins which can only be coagulated with the greatest difficulty. Such a protein was described by Zanetti [1897] who found that, by the addition of a large volume of alcohol to the concentrated filtrate of ox blood, from which all ordinary protein had been removed by careful acidification and boiling, a substance was precipitated which gave all the usual protein reactions. Zanetti held that this body belonged to the class of the mucoids. K. A. H. Mörner [1902] and Eicholz [1898] have also discussed the presence of a mucoid substance in the blood, but believe that it is an artifact. Howell [1906], however, decided in favour of the serum containing a protein not coagulable by heat—Chabrie's albumone—a substance which is neither a proteose nor a peptone. Still more recently Bywaters [1909] also reached the conclusion that the "proteose" described in blood is in reality seromucoid. He maintains that the above-mentioned behaviour of the proteoses present in blood agrees with the characteristics of this body.

As the result of his experiments with hemielastin—an elastin proteose—Borchardt [1907] came to the conclusion that proteoses of

the food could be found not only in the blood but in certain of the tissues. After feeding with hemielastin (artificially prepared) he found evidence of its presence in the blood, liver, spleen, muscle, stomach and intestinal wall. Later he fed elastin itself and thus allowed the elastin proteose to be formed in the normal course of digestion and confirmed his observation that the proteose could be detected in the blood and the tissues. It was not confined to the portal blood, but was also found in the systemic (carotid) blood. Abderhalden and Ruehl [1910] were quite unable, using every possible precaution, to confirm this work of Borchardt. They found no trace of the hemielastin in either blood or tissue. They stated further that as a source of protein supply elastin could not be considered as it was very poorly absorbed. They admitted that it might, however, have a slight protein sparing action. Körösy [1908], using the most modern and careful methods, and Howell [1906], using a method of dialysis where all protein was held back, were quite unable to detect the presence of proteose and peptone in the blood, even at the height of digestion. Abderhalden and Pincussohn [1909] have further found that, after the injection of peptone, a ferment appeared in the plasma capable of decomposing the injected material. If peptone were a normal constituent of the blood it would be expected that the ferment would also be constantly present, but such is not the case.

The conclusion must be reached that there is no very decisive evidence in favour of the presence of either proteose or peptone in the blood. If it be present, it must be merely in traces.

Fate of Protein after Absorption.

If the view be untenable that the whole of the digested protein enters the organism from the intestine in the form of proteoses and peptone, in what form or forms does it enter? Until recently a very marked difference of opinion on the question existed. One set of workers claimed that immediately after absorption the products of digestion were synthetized to a coagulable protein, whereas the other set maintained that the absorption took place in the form of very simple protein products—either as simple amino acids or groups of these—and that it was in this form that protein nitrogen was conveyed in the blood stream, allowing each tissue to choose for itself the particular amino acids required. As Leathes neatly put it "the proteins circulating in the blood are a currency which is not legal tender".

Abderhalden, among the modern workers, was the most active in

upholding the view that a synthesis took place in the intestinal wall immediately after absorption, and that therefore all the protein material was sent on into the animal organism in the form of coagulable protein. Abderhalden and his co-workers held further that the protein, which was formed, was a neutral protein, probably serum protein. They relied for their evidence very largely on the fact that the decomposition products of the protein had not, in their opinion, been clearly demonstrated to be present in the blood. Abderhalden, however, admitted that the methods in vogue for the estimation of small amounts of amino acids were very unsatisfactory. This was certainly the case.

If there be no immediate synthesis to protein, the building material must be carried in a soluble form in the portal blood, and then distributed in the blood stream to the tissues of the body in order that these may take the material they require to satisfy their immediate wants.

The great difficulty in reaching a final settlement of this question arose from the fact that, on account of the rate at which the blood flows through the intestinal vessels, the absorbed material is removed extremely rapidly and is only present in small amount; a given amount of blood at a given moment contains only mere traces of the material to be tested for. Even the ingestion of urea, provided time be allowed for its absorption, does not, as McLean and Selling [1914] point out, materially alter the constant between the amounts of urea found in the blood and in the urine. Further, it must be remembered that the digestion and setting free of the soluble digestion products in the intestinal canal is not explosive in character but is a gradual process thus limiting the amount of material available for absorption in a given time. A number of estimations of the rate of intestinal blood flow have now been made. Cybulski [1906] measured the rate of flow through the portal vein of a dog which weighed 9·5 kilos. and found the rate of blood flow to be about 9000 c.c. per hour or about 150 c.c. per minute. Burton Opitz [1908] found in his experiments on the rate of the blood flow in the portal vein of dogs, that the mean amount which passed along this vein was 594 c.c. per kilo. animal per hour, which works out about a third slower than the flow as estimated by Cybulski. Now Pflüger [1899] showed in a series of experiments in which cats were fed with lean meat that the maximal rate of absorption of the protein for an animal of one kilo. in weight was 1·14 gm. protein per hour. Pflüger [1909] maintained that this was a good maximum for human beings as they cannot digest protein at

the same rate as animals. In support of this statement he cited the case of a dog of 30 kilos. which digested 2500 gms. of meat in twenty-four hours, whereas a man of twice the weight could hardly manage half this amount with comfort. If then the rate of absorption be taken at 1·14 gm. protein per kilo. per hour, and using Burton Opitz's figures, the 1·14 gm. is contained in 594 c.c. of blood, thus the percentage concentration of protein digestion products in the blood is ·19. If, on the other hand, we use the figures obtained by Cybulski the result is still lower, for here we have the 1·14 gm. dissolved in some 950 c.c. of blood, i.e. a concentration of ·12 per cent. Other workers, Bergmann and Langstein [1905], for example, put the amount to be looked for as low as ·005 per cent. of nitrogen. Bang [1916, 1, 2], who also investigated this question, came to the general conclusion that the mean value for the non-protein nitrogen of blood was 25 mgm. per cent. (physiological variation 19-30 mgm.). Amino acid nitrogen averaged 12 mgm. per cent. He further [1916, 3] came to the conclusion that the absorbed amino acids travelled mainly in the plasma. The amount of actual ammonia found in normal blood is very small. Henriques and Christiansen [1917, 1, 2] put the ammonia content of normal blood as low as 0·27 mgm. per cent. and stated that there was no marked increase after an abundant protein meal. In this connexion the very curious observations of Folin, Rohde [1915] and others that in blood drawn and kept aseptically there is a spontaneous liberation of ammonia, are of interest. Rohde concluded that there was a labile non-dialyzable constituent normally in the blood. But the difficulty does not end here as in the first place we know only a fraction of the substances obtainable by the digestion of proteins, even leucine, the most abundant amino acid, common to most proteins, is present on the average only to the extent of 20 per cent., and in the second place this search for digestion products is being made in a fluid which already contains some 3 per cent. of nitrogen in the form of coagulable protein, and about ·03 per cent. nitrogen in the form of nitrogen non-precipitable by tannic acid. If a limited amount of blood only be available unless the search for products of digestion be made in the portal blood the chances of detecting them must be small, as, in addition to the liver acting as an efficient filter and deamidizing organ, the tissues fix a large percentage of the circulating non-protein nitrogen at a very rapid rate.

In spite of all these difficulties, a certain amount of evidence was obtained which supported the contention that the simple products of

digestion are to be found in the blood, more particularly in the portal stream at the height of digestion. Cathcart and Leathes [1905] attempted to demonstrate the increase of the non-precipitable nitrogen (by tannic acid) in the blood following absorption, by performing a perfusion of the isolated intestine in which the same blood was repeatedly used, and in which, therefore, the products from absorption would presumably accumulate. Owing to the fact that the mucous membrane of the intestine ceased to function this method had to be abandoned. By utilizing the whole animal and allowing the absorption of peptone, proteoses or tryptic digestion products to take place from a limited portion of the intestine, a constant increase of the non-precipitable nitrogen in the blood was found. It was definitely shown that the whole increase was not due to urea and ammonia. Only some 15 per cent. of the nitrogen, which was absorbed, could be accounted for by the increase of the non-precipitable nitrogen in the blood, and when the liver was also examined about another 15 per cent. was found to be retained there. Further, an attempt was made to detect the presence of an increase in the coagulable protein (which was to be expected if the immediate synthesis hypothesis were correct), but no such increase could be detected although full allowance was made for the dilution of the blood by fluid absorbed from the intestine. If the amount of nitrogen excreted in the urine during the period of absorption were also considered in addition to the non-precipitable nitrogen found in the blood and liver, the total amount of nitrogen which had disappeared from the lumen of the intestine could be largely accounted for (over 70 per cent. in one experiment).

Bergmann and Langstein [1905] examined the portal and systemic blood of well-fed dogs for total nitrogen and non-coagulable nitrogen, and found that there was always a slight gain in the non-protein nitrogen—"residual" nitrogen—after a meat meal. The percentage amount of non-protein nitrogen of the total nitrogen of the blood varied between 7·7 and 14·7 with an average of 10·7. This coagulation method cannot be regarded as satisfactory as the non-coagulable nitrogen cannot with certainty be pronounced to be simple amino acids, polypeptides, etc., in the light of the statement that non-coagulable proteins are sometimes present in the plasma. Bergmann [1905], utilizing the β-naphthalene sulphochloride method introduced by Fischer and Bergell, stated that he was able to obtain from the blood of a patient suffering from acute yellow atrophy a product which crystallized out, but which he was unable to identify. He also obtained evidence of the presence of bodies, which could unite with the

β-naphthalene sulphochloride, but which he was also unable to identify, in the blood of dogs killed after an abundant flesh meal. Further, in the blood and expressed juice from muscles and liver of an animal which had fasted he was unable to demonstrate the presence of any material which would combine with the β-naphthalene sulphochloride. Howell [1906] used the same method, but, by an ingenious device, he got rid of the serum proteins without the risk of losing part of his amino acids in the coagulum formed on heating. He enclosed the blood taken from fed and fasted dogs in sacs of collodion and dialyzed it against distilled water. Amino acids and substances as complex as the proteoses will pass through this membrane. Howell found evidence of amino acids being present in the portal blood of well-fed animals in greater amount than in the systemic blood. Even after fasting for fifty hours he stated that a positive amino acid reaction might be obtained from the blood. He also found that the lymph collected from the thoracic duct after a meal gave a positive reaction. Although Howell obtained these positive reactions he did not obtain a pure crystalline body but a sticky substance which he could not identify.

Hohlweg and Mayer [1908] also examined the question of this "residual nitrogen". They found a constant increase of this residual nitrogen in the blood of fed dogs above that taken from the fasting animal. In fasting there was present in 100 c.c. serum 0·0525 gm. total residual nitrogen and 0·0384 gm. urea, whereas in the digesting animal there was present in the same amount of serum 0·0788 gm. residual nitrogen and 0·0567 gm. urea. In both cases urea formed about 73 per cent. of the total residual nitrogen. Using the tannic acid precipitation method they found an increase in the non-precipitable nitrogen in the blood of the digesting animal as compared with the fasting animal. The increase amounted to ·007 gm. nitrogen in 100 c.c. blood which equals, as they have calculated, ·0705 gm. leucine, or much less glycine.

Abderhalden and his co-workers produced a certain amount of indirect evidence against the view that the nitrogenous products after absorption pass on to the tissues in a non-protein form, but no direct evidence of value in favour of the view that immediate resynthesis took place. Abderhalden, Funk and London [1907] stated that they were quite unable to demonstrate by the most modern chemical methods that either proteoses or amino acids were present in the blood of dogs with Eck's fistula when fed on different diets. Certainly a rise of ammonia in the blood was to be expected as the liver was cut out of the

circulation, but no such rise was found. They believed their experiments to be direct evidence in favour of the hypothesis of immediate resynthesis. Gliadin was one of the proteins fed, but even under what must be considered very favourable experimental conditions, they could not account for the fate of the excess of glutamic acid—not even in this experiment could they detect a rise in the ammonia content of the blood. Abderhalden and London [1907], as the result of a further series of experiments on a dog with an Eck fistula, reiterated the opinion that the experimental results—nitrogenous equilibrium maintained on fully digested meat products—afforded strong support for immediate resynthesis. These authors further attempted to support the hypothesis by investigating the variation in the nitrogen content of the intestinal wall, but they failed to get conclusive evidence [1909], [1910]. In their experiments they used control pieces of intestine from the same animal on which the experiment was carried out. London [1909, 2] also tried to prove that resynthesis of protein took place immediately after absorption, but the results which he obtained were negative. He argued that, if the intestinal mucosa were actively concerned in resynthesis, evidence of the resynthesis should be obtained by a comparative chemical analysis of the mucosa during hunger and at the height of digestion (of gliadin). His control animals gave a nitrogen content, for 50·1 gms. dried mucosa, of 3·15 gms. nitrogen, and 0·75 gm. glutamic acid hydrochloride and his digestion mucosa, for 50 gms. dried substance, 3·10 gms. nitrogen and 1·40 gm. glutamic acid hydrochloride. Thus, even at the height of digestion, he was unable to prove that there was any accumulation of nitrogenous material. This is, of course, no proof that such a synthesis does not take place, as it is possible, although highly improbable, that the synthetic product is very rapidly formed (constant synthetic action) and just as rapidly removed. It does not, on the other hand, support the immediate resynthesis hypothesis. Körösy [1908] carried out a large number of absorption experiments under different conditions, using a method very similar to that followed by Cathcart and Leathes. He pronounced, however, in favour of immediate resynthesis, on the grounds that in dogs with their circulation restricted to the intestine the non-precipitable nitrogen (by the tannic acid method) in relation to the total nitrogen of the blood is not increased after a protein meal in greater amount than that which is found in fasting. He was unable to detect any free amino acids or proteoses in the blood, and this he regarded as an additional argument for immediate resynthesis.

Cohnheim [1902], on the other hand, produced some evidence which distinctly favoured the view that the absorbed protein material travelled in the blood in the form of amino acids. He carried out his experiments on the intestine of the Octopus and Eledone moschata. He introduced solutions of peptone into the isolated gastro-intestinal tract, which he floated in oxygenated blood. Not only was he able to prove that absorption took place, but that amino acids were absorbed, at any rate under the conditions of his experiment, as he was able to isolate from the blood at the conclusion of the experiment, which lasted twenty hours, leucine, tyrosine, lysine, arginine and ammonia. When he carried out similar absorption experiments on the intact animal, he was unable to detect these amino acids in the blood. He repeated this work with isolated intestine [1912], using Ringer's solution as the bathing fluid, and in each experiment found an increase in the nitrogen content of the bathing solution, mainly in the form of ammonia. In no case were the amino acids used recovered from the Ringer solution.

In 1912, however, new light was thrown upon this most difficult of problems by the ingenious work of Folin and later of van Slyke. The results and deductions made from these experiments seemed to be fully completed and rounded off by the still more ingenious methods of Abel. His results were so dramatic and convincing that even the leading protagonist of the immediate synthesis hypothesis capitulated.

Folin and Denis [1912, 1] were the first to publish results dealing with the application to the blood and tissues of Folin's new micro methods for the determination of total nitrogen, urea and ammonia nitrogen. They took samples of blood and muscle (gracilis) and then introduced into a ligatured loop of the intestine solutions of urea, glycine, egg albumin or pancreatic digest; samples of blood were withdrawn at varying intervals during the experiment, and at its close the gracilis was removed from the other leg. The determination of the total non-protein nitrogen and the urea in the samples thus obtained showed that there had been a rapid absorption of the urea from the intestine, that it had entered into the blood stream unchanged and had actually accumulated in the muscle. Results of the same order were obtained with glycine, egg albumin and pancreatic digests. Later [1912, 3], by continuing the experiments over a longer period, they showed that some of the absorbed glycine was converted into urea. They obtained similar results with alanine and peptone. They laid stress on the conclusion that the power of deaminization was not confined to the liver but was characteristic of all tissues. Folin and Denis

[1912, 2] further showed that the ammonia found in the portal blood came mainly from the large intestine and that the injection of pancreatic digest, glycine or asparagine caused no increase in the ammonia of the portal blood. In connexion with the question of absorption Folin and Lyman [1912, 1, 2] also stated that a certain amount of absorption took place from the stomach and from the large intestine.

Van Slyke's work was the result of the elaboration of his method [1912, 1] for the determination of amino acid nitrogen. It is a definite improvement on Folin's method as the amino acid nitrogen was directly determined. Van Slyke and Meyer [1912] found that the blood of normal dogs which had fasted for some twenty hours contained 3 to 5 mgm. amino acid nitrogen per cent., and these quantities were much exceeded when alanine was injected into the small intestine. The amino acid content of the blood five hours after a meal of 1 kilo. meat was practically doubled in amount. They also found that if the alanine were injected directly into the blood stream that it disappeared very rapidly from the circulation, only a relatively small part being excreted in the urine. In a later paper [1913, 1] they elaborated these experiments and demonstrated that the amino acid content of the tissues was increased as the result of the intravenous injection of amino acid. They found, however, that there is apparently a saturation point which sets the limit to absorption, as they were never able to obtain greater saturation of muscle than 80 mgm. per cent. and for the liver 125 to 150 mgm. per cent. When they increased the duration of the experiment [1913, 2] it was found that the liver was able to desaturate itself in the course of two or three hours, whereas the amino acid content of the muscle remained practically unaltered during this period. The disappearance of the amino acid from the blood was accompanied by an increase in the amount of urea. Curiously enough they found [1913, 3] that the amino acid content of the tissues of dogs fed on a protein rich diet was not greater than the amount found in some of the starving dogs.

Abel [Abel, Rowntree and Turner, 1914], by his method of vividiffusion in which a large fraction of the animal's blood could be passed through a dialysing apparatus and then returned to the body, after having lost its diffusible constituents, showed definitely [in 1913] that amino acids could be isolated in considerable quantity from the dialysate. The general conclusion that amino acids existed in the blood stream has received repeated confirmation. Thus Abderhalden [1913] demonstrated that even by the older methods of precipitation, if the quantity of blood used for examination were large enough, a

great variety of amino acids, proline, valine, leucine, alanine, glycine, aspartic and glutamic acids, arginine, lysine, histidine and probably traces of tryptophan could be obtained. Delaunay [1913, 1, 2, 3] found that following either a meat meal or the injection of a solution of amino acids into the intestine there was a definite rise in the blood content of amino acids most marked in the case of the portal blood. The urea content of the portal and systemic blood was equally increased. György and Zunz [1915], using the van Slyke method to investigate the amino acid content of the blood of normal dogs, found that it was remarkably constant, undergoing but little change as the result of the diet unless it were very rich in protein.

The Nature of the Absorbed Material.

There is no evidence yet available which definitely settles the question as to the form in which *all* the protein digest products reach the tissues. It is clear that a large percentage apparently reaches the various organs in the form of amino acids via the blood stream; it is also clear from the work of Howell [1906], Abderhalden, Lampé and London [1913] and Hendrix and Sweet [1917] that there is quite a marked assimilation of amino acids by way of the thoracic duct. Hendrix and Sweet, for example, have shown that if the amino acid content of the blood and thoracic lymph be compared, in the case of starving dogs the blood contains more than the chyle, whereas after the injection of milk, peptone or amino acids into the intestine, although there is an increased amino content both of the blood and the chyle, it is relatively more marked in the case of the thoracic lymph. They suggest that the apparent selective absorption by the blood is merely due to the fact that the amount of blood flowing through the intestine greatly exceeds the lymph flow. Again there is the work of Nolf [1907, 1] and Abel, Pincoffs and Rouiller [1917] where it is maintained that part of the protein may be absorbed as proteose, and again the suggestion of Magnus Levy [1907] that a fraction of the protein may, under quite normal conditions, be taken up unchanged, and finally the statement originally made by Hofmeister that the leucocytes in all probability play an important rôle in the absorption of protein.

The fact that an animal can subsist on digest products or even a mixture of purified amino acids is of course fair evidence that the complete digestion of protein is not incompatible with physiological processes, for a limited period at least. But it must not be forgotten that the organism has a very high factor of safety, it will tolerate

maltreatment in feeding for long periods, just as it will stand total absence of food for many weeks. These experiments are not absolute proof that the whole of the protein molecule is reduced, as a matter of routine, to simple products. Yet the reduction, of the greater part at least, of the protein molecule to these simple forms does seem to be a logical necessity. The body tissues are built up of many different types of protein each of which requires its own special supply of food material for growth and to make good ordinary wear and tear. Also the protein ingested as food is of many types, animal and vegetable, and of varying quality and value to the organism.

This is the strongest argument, to my mind, against the hypothesis which was, until recently, so strongly held by Abderhalden and others that the synthesis of these products into protein took place immediately after absorption. This would undo all the benefit gained by digestion for there would be a pabulum of uniform composition circulating in the body fluids for the nourishment of tissues with very divergent needs. No doubt the intestinal tissue cells possess, as Pflüger [1909] maintained in a most interesting paper in which he combated this immediate synthesis hypothesis, the power of synthetizing protein material, but it is in all probability no more and no less active than any other collection of tissue cells. In the same way the great majority, if not all, cells possess catalytic powers.

The general body of opinion at the moment seems to favour the view that the bulk, many workers would definitely state all, of the protein is digested in the intestine as far as the simple abiuret substances and is then absorbed and distributed throughout the body in this form. But even one of the ablest of the modern workers in this field, van Slyke [1917], is apparently nonplussed by the fact that the liver seems to exercise a perfectly unlimited control over the distribution of the amino acids after absorption. Evidently there is no selective action of any kind, the liver indiscriminately absorbs to saturation point the amino acids from the portal blood. The same apparent lack of discrimination may also be ascribed to the other tissues; they take what they can. Is this apparent indiscriminate seizure due to the fact that, in the great majority of instances, the optimum condition of the cell content is rarely achieved, that the capacity for storage has not been fully satisfied? The difficulty about the solution of this problem is great as there are at present no means by which we can differentiate between the amino acids which arrive from without and those which are present in the tissue as the result of catabolic activity. Although the absorption by the lymph channels

may not account for a large percentage of the amino acids absorbed, still the fact that it takes place does ensure the tissues receiving a certain supply of amino acids direct. And the nitrogen minimum requirement is low (see p. 75).

As regards the statement that the protein is absorbed in part in the form of proteose and peptone, the fact that these substances have never been actually isolated or even unequivocally detected in the blood is fair, although not of course irrefutable evidence, that such an absorption must be remarkably small in amount. The detection of small amounts of proteose actually in the tissues has but little bearing as evidence; it might have been formed as the result of intracellular catabolism. The suggestion that part of the protein may be absorbed unchanged is difficult either to prove or refute. Unless the protein had some very highly specific quality it would be almost impossible to detect it in the blood. It is, of course, a generally accepted fact that unchanged protein can be shown experimentally to be absorbed from the intestine, but there is at present no definite evidence as to whether this is a normal action in the course of ordinary digestion. Finally the part played by the leucocyte is still undecided (see p. 35). There is, of course, absolutely no doubt about the post-prandial leucocytosis, but the evidence offered in support of the view that these cells act as carriers of protein is very unsatisfactory.

The Hypothesis of Freund.

Freund and his pupils hold that during or after absorption of the protein digest products they undergo some kind of polymerization which is absolutely essential before synthesis of protein can be effectively carried out by the tissues. Toepfer [1906] found that, if the liver of an animal were perfused with its own blood, there was no increase in the blood of any decomposition products even after the addition of protein to the blood used in the perfusion. If an addition of Witte's peptone were made there was a slight increase in the amount of coagulable nitrogenous products at the expense of the proteose. If, on the other hand, the intestine as well as the liver were left in the circulating area an increase of decomposition products in the blood could always be detected. He came to the conclusion that both the liver and intestine were necessary to the proper breakdown of proteins. Freund and Toepfer [1906] continued the investigation. They perfused the liver and intestines of two fasting dogs with (1) their own blood, (2) with the blood of a well-fed animal. They found that in both cases there was an increase in the nitrogenous decomposition products, but

that the increase in the case of the perfusion with the blood of a well-fed animal was about twice that when the blood from the fasting animal was utilized. Freund [1907] later came to the conclusion that the liver played a very essential part in the breakdown of protein, but that before this action could be evoked the protein material must have passed through the intestinal wall. Even in starvation he held that the various autolytic products must first be excreted into the intestine and then be reabsorbed and altered in some mysterious fashion before they could be utilized. He believed, like Abderhalden, that the protein digestion products travelled in the portal stream in a coagulable form—chiefly as pseudoglobulin. Freund's experiments are extremely difficult to understand, as not only is his explanation very obscure, but the experimental data which he offers are difficult of interpretation even in the light of his own hypothesis.

Körösy [1909, 1910] tested the hypothesis of Freund by using dogs in which practically the whole of the intestine was cut out of circulation. He then injected foreign serum into the blood stream in the expectation that under these conditions, if the intestinal wall did perform certain essential preparatory functions, the protein would appear in the urine unchanged. Protein was either absent or appeared only in traces, so that, if we accept Körösy's interpretation of these experiments, the preparatory action of the intestine advocated by Freund cannot be very essential. In the course of his experiments Körösy made the curious observation, previously recorded by Slosse [1890], that the mere cutting of the intestine out of the circulation leads to the appearance of a certain amount of protein in the urine.

Abderhalden and London [1909] were unable to show that any excretion into the intestine took place after the subcutaneous injection of protein. In their experiments they used animals with intestinal fistulæ, where any excretion, even in traces, could be comparatively readily detected under fairly normal conditions. Still, a certain amount of independent evidence exists, which might be regarded as lending support to this contention of Freund. Thus London and Polovzova [1908] found that certain nitrogenous bodies were excreted into the intestine high up and were absorbed again lower down. Further, Reach [1909] found that, if he perfused a liver with a mixture of blood, Ringer's solution and a protein containing iodine, a definite retention of the iodine-containing protein took place in the liver. He believed that the slight degree of proteolysis of the perfused iodine protein compound which occurred supported, to a limited extent, the hypothesis of Freund. Aberhalden and Slavu [1909] also found, after

the subcutaneous injection of certain compounds of iodine and polypeptides, that iodine appeared in the intestine and was excreted in the fæces, in other words, that a definite excretion into the intestine had taken place.

A somewhat similar hypothesis to that of Freund was put forward for the carbohydrates by Croftan and may be mentioned here as it also bears on the existence of the possibility of excretion into the intestinal canal. Croftan [1909] stated that dextrose by its passage through the intestinal mucous membrane underwent some alteration which rendered possible its polymerization into glycogen in the liver. He further stated that, if this passage were omitted, and the dextrose injected directly into the mesenteric vein there was no increase in the glycogen of the liver, but that an actual excretion of sugar from the blood into the lumen of the intestine occurred. He held that the experiments of Grube, who showed that perfusion of the tortoise liver with dextrose could give rise to glycogen, were without value as they were carried out on a cold-blooded animal. Pflüger, it may be noted, held that this objection was not valid. Grube [1909], who repeated Croftan's work, was quite unable to confirm it. His explanation of the variation which Croftan found in the glycogen content of the liver is both plausible and interesting. Fischer and Moore [1907] had also observed this excretion of carbohydrate into the intestine, but the conditions under which it occurs are more or less pathological.

Plastein Formation.

Intimately connected with the preceding work on protein regeneration are the curious observations on the so-called plastein formation. This work originated in the experiments of Danilevsky and Okuneff [1901], who showed that if rennin were brought into contact with a solution of proteoses a precipitate was produced. They regarded this precipitate as a resynthetized protein. Kurajeff [1904] observed the same formation of a precipitate when papayotin solutions were brought into contact with proteose solutions. He also stated that he could obtain the formation of a coagulable protein from his plastein proteoses, if they were brought into contact with the gastric or intestinal mucous membrane. Nurnberg [1904] found that the plastein formation was not limited directly to the action of the gastric rennin, for if autolyzed tissue extracts were brought into contact with protein solutions a precipitation resulted. This work was confirmed by that of Grossmann [1906]. Savjaloff [1901] also investigated this precipitation reaction. He came to the conclusion that in the course of the digestion in the

gastro-intestinal canal a substance was formed which, after absorption, was capable of being coagulated later when and where required. He stated that if the proteose solution were fractionated in the manner described by Pick, and if the individual fractions were then treated with the enzyme, no precipitation resulted, although the same proteose solution not fractionated gave the precipitation quite readily. He was firmly convinced that the reaction was a true synthetic one. He further held that the substance formed was a true substance of constant constitution. He gave to the substance the name plastein.

On the other hand, Lavroff and Salaskin [1902] held that there was no reason why the precipitate should be accepted as an entity—a regenerated peptone. They believed that it was a mixture and suggested that "rennin proteose" should be the name given to it. They obtained it from the different proteose fractions. Savjaloff, later [1907], restated his position and again maintained that the formation of plastein was a true synthetic process, and that it could only be demonstrated in strong proteose solutions. He thought that in all probability it was evidence of a reversible action of pepsin. He regarded it as an assimilation product of first-rate importance, the intermediate product between digested protein and the formation of blood proteins. This change took place, he believed, immediately after absorption. He was convinced, that when proteins have reached what may be called the plastein level in enzymic degradation, they are of uniform constitution. Lavroff [1907, 1, 2] certainly obtained the precipitation as other authors have done, and he called the substance shortly "coagulose". He found that it could be produced from digestive products from which the hexone bases had been removed, and that the product which was formed, contained no such bases. It was also formed from material in which these bases were present, and in this case the coagulose contained them. He was not therefore inclined to regard them as specific substances. In a later paper [1909] he demonstrated that, by the peptic digestion of caseinogen, two series of "coagulose" substances were formed, one conforming to a proteose type, the other to a polypeptide type. Henriques and Gjalbak [1911] also found that pepsin could produce plastein formation from peptic digests and that the composition of the plastein varied according to the degree of splitting of the digest products used as substrates. In a later paper [1912] they summarized their results. They believed plastein to be a synthetic product and that it was no simple substance but in all probability a mixture of proteoses.

Sacharoff [1903] held, that this plastein formation was not synthetic

in origin at all, that it was only an intermediate substance in the process of digestion, precipitation taking place simply because the physical conditions for solution were not suitable. Bayer [1904] also stated that, in his opinion, the substance formed was no true synthetic product, and thought that its protein-like character was probably due to impurities. He believed that it was a member of the so-called peptoid group of Zunz, and that it was a body therefore of comparatively simple constitution. Herzog [1903] and Volhard [1903], maintained that this plastein formation was not the result of the action of rennin at all, but might be regarded as another example of the reversibility of reaction of proteolytic ferments (Herzog) or of pepsin (Volhard). Rachoczy [1911] thought that plastein formation was a function of all proteolytic enzymes. Glagolew [1911] demonstrated clearly that the ferment played a real part because if the enzyme were killed no plastein formation took place. He held the formation was a true reversible reaction in which three factors played important parts: (1) amount of ferment, (2) concentration, and (3) reaction of, the substrate. London [1911] also showed that a similar coagulation took place in the chyme removed from the intestine twelve or more hours after feeding with casein. It had the curious property of becoming fluid and then, under suitable conditions, again coagulating.

The question as to (1) whether this substance is a new synthetic product differing from the protein from which the proteoses are derived, or (2) whether it is merely a resynthesis of the original protein from which the proteoses are obtained, or (3) whether it is no synthetic product at all but merely a substance—a digestion product—on the road to complete solution is not definitely settled. The work, however, of Levene and Van Slyke [1908, 1909] would incline one to the view that the substance belonged more to the proteose than the true protein group of bodies. Levene and Van Slyke carried out a complete hydrolysis of the material, with subsequent isolation of the amino acids, and found that it contained at least thirteen amino acids. It must, therefore, be regarded as a fairly complex body. The evidence [1909] obtained by the investigation of the viscosity also pointed rather to its proteose than its protein nature, precipitation taking place owing to mere difficulties of solution, as Sacharoff had already suggested.

Some curious and rather indeterminate experiments on the reversible action of digestive ferments have been carried out and may be mentioned here. A. E. Taylor [1907], for example, stated, that if the concentrated products of the tryptic digestion of protamine were

subjected to the further action of fresh trypsin for five months reformed protamine could be obtained. It is true that the amount of resynthetized product was not large, as from the digestion products of 400 gms. protamine he obtained only 2 gms. of synthetized material. The trypsin employed was obtained from the liver of clams. In a later paper [1908] he confirmed his original finding and adduced additional experimental evidence. Brailsford Robertson [1907, 1908] found that by the action at 40° C. of a concentrated solution of pepsin on an acid concentrated solution of the products of peptic digestion of caseinogen a substance was precipitated within a few hours which was identical in properties and phosphorus content with a substance related to paranuclein. This material was only formed by the action of the pepsin on the caseinogen digest, since if both these substances were kept separate under similar experimental conditions no precipitate was formed. Robertson and Biddle found [1911] that variations in composition of the resulting product occurred, depending (1) on the temperature at which the experiment was carried out and (2) on the concentration of the peptic digest used as substrate.

Synthesis in the Gastric and Intestinal Mucous Membrane.

Closely allied to this work on plastein formation is that of Hofmeister and his pupil Glaessner, some of whose observations were made previous to the publication of the work of Danilevsky and Okuneff. Hofmeister [1885] stated that if proteoses were left in contact with the gastric mucous membrane they were converted into protein. In his experiments he divided the stomach of a dog, which had been killed at the height of digestion, into two approximately equal parts. One of these parts he immersed at once in boiling water, and the other he placed for two hours in an incubator. He then estimated the amount of proteose and peptone obtainable from each part employing the biuret reaction colorimetrically for the purpose. A diminution, even a complete disappearance, of proteose and peptone was observed in the incubated half. He concluded, therefore, that the proteose and peptone had been converted into protein through the agency of the gastric mucous membrane. He found that, if he had previously warmed the part of the stomach to be incubated to 60° for a short period, it lost its synthetic power. Glaessner [1901] confirmed these experiments of Hofmeister using more exact methods. He killed dogs three to fourteen hours after a heavy meat meal, and immediately removed the stomach, which he carefully freed from its contents, then divided into two approximately equal parts. In one part the pro-

teose content was at once determined whilst the other part was placed in a moist chamber at 40°. He found, like Hofmeister, that there was a very marked diminution in the amount of proteose to be obtained from the incubated part and at the same time no increase in lower digest products. He also concluded that a true resynthesis of the proteose to protein had taken place—a synthesis which began soon after the commencement of digestion and which reached its maximum between the fifth and sixth hour and then gradually decreased. He thought that the change was brought about by a proteo-synthetic enzyme but he did not think that rennin played a part. Other observers, however, have criticized these observations of Glaessner and have offered other explanations of his results. Embden and Knoop [1903] repeated Glaessner's work using intestinal mucous membrane (a tissue which had also been previously used with success by Hofmeister). They could find no trace of protein resynthesis, either in the natural intestine or in an intestine freed from trypsin, by previous ligature of the pancreatic duct, to prevent digestion of any newly formed material. At the same time they found no evidence of the further breakdown of the proteose. Cohnheim [1902] believed that the difference found by Glaessner between the amounts of proteose present in the two parts of the stomach depended, to a certain extent at least, on the fact that fresh tissue coagulated only with difficulty. It is questionable if this argument is valid. Salaskin [1902] suggested that the changes which were observed might depend on the alterations which take place in the cells during the resting period—that the apparent synthesis was nothing more nor less than the normal cell restitution. Neither of these series of experiments is very convincing, but on the whole the evidence from digestion and absorption experiments generally does not indicate the occurrence of any marked resynthesis in the stomach wall, at least during normal digestive processes. Glagolew [1911, 1913] has also investigated the question and found very definite variations. These were largely due to very simple alterations of various factors among which autolysis played a leading part.

The Rôle of the Leucocyte.

Another view of the process by which protein digestion products are dealt with after absorption has been put forward by Hofmeister [1885]. He believed that the peptone after absorption was taken up by the leucocytes and then either through their own agency or through that of the adenoid tissue it was converted into protein. This contention

was largely based on the marked leucocytosis which was found to occur after a meal and not on the direct estimation of the contents of the leucocytes. In support of this hypothesis of Hofmeister, Pohl [1888] found that during digestion, in addition to the postprandial leucocytosis, there was an excess of leucocytes in the mesenteric veins as compared with the mesenteric arteries. Paton, Goodall and Gulland [1903] showed that there was no detectable difference between the number of white cells in the veins and the arteries. They, however, confirmed the postprandial leucocytosis and showed that the most marked percentage increase occurred in the lymphocytes. There was also some increase in the polymorphonuclears but practically no change in the number of the eosinophiles. The maximum increase in the number of the leucocytes took place about four hours after food. Brasch [1912] found the leucocytosis, which mainly consisted of lymphocytes although all cells sometimes increased, reached its maximum four to ten hours after the taking of food. All classes of food-stuff, protein, carbohydrate and fat bring about leucocytosis. Paton and Goodall [1905] later demonstrated that the leucocytes did not arise, as Hofmeister believed, in the intestinal lymphatic tissue but in the bone-marrow. Erdély [1903] also worked at this problem and found that the intestinal wall was richer in leucocytes after a meal than after a period of starvation. He believed that alterations in the nature of the diet brought about variations in the nature of the leucocytosis. Cramer and Pringle [1908] have also supported the hypothesis that the leucocytes played a very important part in the assimilation of the protein food products from the intestine, and Cramer [1908] believes that even in the case of protein introduced parenterally assimilation is the result of the action of the leucocytes. Schittenhelm, Weichardt and Grisshammer [1912] have also shown that parenterally introduced protein, peptone, etc., is followed by a temporary but marked leucopenia and then in the course of an hour or two a leucocytosis appears which may persist for days. Pavy [1908] held that the whole conversion of the food protein into tissue protein was brought about by the lymphocytes. He maintained that the products of protein digestion were resynthetized at the seat of absorption by the lymphocyte growth and that the lymphocytes in turn were resolved by autolysis into the proteins of the blood and in this way the fresh food material was brought within reach of the tissue cells.

None of these workers support their hypothesis by any conclusive observations on the variation in constitution of the leucocytes before

and after food, although Cramer and Pringle [1908] did obtain some evidence that the composition of Peyer's Patches differed before and after a meal. There is no doubt about the postprandial leucocytosis, but it is not yet proven that these leucocytes are engaged in the manufacture of the new food for the tissues. Halliburton [1909], who pointedly criticized this leucocyte synthesis theory, maintained that the number of the lymphocytes available was not commensurate with the work to be done. He calculated that a man of eighty kilos. had about four kilos. of blood of which some forty per cent. was in the form of corpuscles, that is about 1600 gms. Now as the ratio of white corpuscles to red is about 1 : 500 it means that about 3·2 gms. of leucocytes are present. Of this amount lymphocytes form at most thirty per cent., and therefore in the blood there would be about one gram of lymphocytes. If this amount were doubled during digestion, "it is difficult to see how two grams of lymphocytes can tackle the enormous burden which every meal must impose upon them". Even using the figures of Gulland, who stated that the rise in the number of leucocytes might be as much as four times, the difficulty in ascribing so large a synthetic action to this comparatively small number of white cells is great, more particularly if, as Pavy supposed, the newly formed protein was liberated by a complete autolysis of the cell in which the synthesis took place.

CHAPTER III.

FEEDING EXPERIMENTS WITH ABIURET PRODUCTS.

The Value of Abiuret Products of Digestion.

INTIMATELY connected with the question of the extent of protein digestion and the form in which the digestion products are absorbed are the feeding experiments with pre-digested protein. These important experiments, first carried out by Loewi [1902], have yielded valuable results. They have clearly demonstrated that a food in which the nitrogen consisted wholly of protein digestion products which no longer gave the biuret reaction, was capable not only of maintaining life but of keeping the animal in a state of nitrogenous equilibrium and even of leading to a certain retention of nitrogen and a rise in weight. An interesting fact was discovered in the course of Loewi's investigations, a fact which disposed of one of the objections to the complete breakdown of protein in the intestinal canal. A calorimetric estimation of 1 gm. of the digestion products used by Loewi in his experiments was carried out by Rubner. This amount was found to yield 4·599 calories, a figure very close to the mean for protein. In Loewi's experiments the rest of the animal's diet was made up of fat and carbohydrate. He found that, if he fed the digestion product with fat alone, no nitrogenous equilibrium resulted, but that this took place as soon as carbohydrate was added. Lesser [1904], who was one of the earliest workers to repeat Loewi's work, was unable to confirm it. Lesser used in his experiments both peptic and tryptic digestion products, but was unable to get a positive nitrogen balance, although the products acted as sparers of protein. In spite of Loewi's bad results with fat alone Lesser omitted carbohydrate from his diets. Henderson and Dean [1903] were the first to use acid hydrolytic products in their experiments. They found that they got nitrogen retention but were not at all certain that it indicated protein synthetic action. As the result of the line of research opened up by the original experiments of Loewi a great number of experiments have been carried out. Abderhalden and

Rona [1904] fed mice on a diet of carbohydrate and different preparations of caseinogen :—

(1) Caseinogen digested for two months with pancreatin. The preparation gave a faint biuret reaction and contained about 15 per cent. of polypeptides.

(2) Caseinogen digested for one month with pepsin-hydrochloric acid mixture, then for two months with pancreatin. The preparation gave no biuret reaction and contained only about 8 per cent. of polypeptides.

(3) Caseinogen hydrolyzed ten hours with 25 per cent. sulphuric acid. The preparation contained no polypeptides.

(4) Unaltered normal caseinogen.

Oil was omitted from the diet as when it was present the mice refused the food. They found that the mice fed with preparations (1) and (4) lived about the same length of time. Mice fed on diet (2) as a rule died earlier than these, but lived longer than mice fed on sugar alone, and mice fed on diet (3) died at about the same time as those fed on sugar alone. Thus it will be noted that the least broken down (15 per cent. polypeptides) of the digestion products behaved most like the normal caseinogen, that next came the preparation with 8 per cent. of polypeptides, and finally the preparation which to all intents and purposes could not be regarded as a food, the acid product with no polypeptides. This evidence is certainly in favour of the hypothesis that certain nuclei are left more or less intact during digestion *in vivo*. But experiments carried out on mice can never be regarded as very reliable unless very large numbers of them are used as controls, as the individual differences in the powers of resistance to diet, starvation, etc., are so great that the results of separate experiments are hardly comparable. Abderhalden and Rona [1905, 1] repeated these experiments on a dog, and found unmistakably that part at least of their earlier work on mice was correct. They found that the biuret free digest could completely take the place of protein in the diet, but that the acid hydrolytic product could not do so. In the digest product used in these experiments about 10 per cent. of the nitrogen was in peptide form. The diet contained both fat and carbohydrates. Abderhalden and Rona offered as an explanation of their negative results with the acid hydrolytic product, that there had been first a complete disintegration of important and necessary compounds, and secondly that racemization of the amino acids had also in all probability taken place. The same workers later [1906, 1] tried to replace the protein in the diet by a mixture of single

amino acids, but the attempt was quite unsuccessful. But little value need be attached to this experiment as many of the amino acids ordinarily found in proteins were absent.

Henriques and Hansen [1904] carried out a series of experiments contemporaneously with much of the German work. They also found that acid decomposition products could not replace protein in the diet although digestion products, which resulted from the long-continued action of trypsin and erepsin, could not only prevent the loss of nitrogen but could even lead, as Loewi had found, to retention. They further found that the loss of nitrogen could be prevented by feeding with the fraction of the digest products which was *not* precipitated by phosphotungstic acid, i.e. the monoamino acid fraction. They also obtained the same result when the products of a tryptic digest soluble in warm 96 per cent. alcohol were used, whereas the alcohol insoluble products could not prevent loss of nitrogen.

Sörensen [1908] has suggested that these positive results of Henriques and Hansen with the monoamino fraction alone were due to this fraction still containing the essential polypeptides which were not precipitated with phosphotungstic acid (Pflaundler [1900] had shown that such exist). Sörensen showed that about 20 per cent. of the total nitrogen of the "monoamino fraction" was still in polypeptide form. In a later paper Henriques and Hansen [1906] showed clearly that although the products of acid hydrolysis of protein could not replace protein in the diet as efficiently as the products produced by the action of enzymes, they were nevertheless excellent protein sparers, a fact already demonstrated by Henderson and Dean. They concluded, however, from another series of experiments that these same digestion products if fed along with a protamine (clupeine sulphate) could bring an animal into a state of nitrogenous equilibrium. Protamine given alone would even seem to exert a certain protein sparing power.

Abderhalden and Oppler [1907] kept a dog alive for thirty-eight days, during which time the nitrogen supply was in the form of an abiuret digest mixture consisting almost solely of amino acids. Abderhalden and Rona [1907] fed a young dog for three weeks on completely digested meat—biuret free—and found not only a retention of nitrogen, which was to be expected under normal conditions with the growing animal, but a distinct increase of weight. Buglia [1912, 1] also found that digest products as the source of nitrogen do suffice for growth provided there is an abundant supply of carbohydrate. Abderhalden [1908, 2] was also able to keep a bitch in nitrogen-

ous equilibrium with fully digested meat during lactation. Even more astonishing was the result of an experiment carried out by Abderhalden and London [1907] on a dog with an Eck fistula, as not only did they get their animal into a state of nitrogenous equilibrium, but it retained a certain amount of nitrogen, although the protein part of the diet consisted wholly of fully digested meat. The body weight of the animal slowly sank, however, during the course of the experiment. Abderhalden and London used this experiment as a weighty piece of evidence in favour of the synthesis of the protein digestion products taking place during the process of absorption. They also stated in conclusion that the liver must not be considered as an organ of absolute importance in protein synthesis. It might be deduced that such experiments as the above negatived the suggestion that the presence of peptide linkages were of considerable importance but as Andersen [1915] has pointed out it is practically impossible to free digests from peptides by ferment digestion. Usually about 10 per cent. peptide compounds are left.

Henriques [1907] returned to the question of the difference in nutritive value which exists between ferment and acid hydrolytic product. He found that ferment digestion products (trypsin and erepsin) heated with 20 per cent. sulphuric acid for six hours in a boiling water bath could keep the animals in nitrogenous equilibrium, and even bring about a retention of nitrogen, yet the same products heated in the same way with the acid for seventeen hours utterly failed to produce this sparing effect. The only difference which he could detect between the two preparations was that after the six hours' acid hydrolysis the tryptophan reaction could be obtained, but that in the seventeen hours' specimen it had disappeared. In this connexion the work of Willcock and Hopkins [1907] is of interest (see p. 86).

Abderhalden also re-examined this question in the light of these results of Henriques. Abderhalden and Frank [1909] completely hydrolyzed horse flesh by boiling with sulphuric acid and then added to the prepared products, before feeding them to dogs, 0·5 per cent. tryptophan. They found it was then possible to keep one dog in nitrogenous equilibrium for twelve days and another for fourteen days. During these periods the body weight remained fairly steady. E. Voit and Zisterer [1910] have worked out the actual relationship between undigested caseinogen and caseinogen hydrolyzed (*a*) by pancreatin, (*b*) by acid, as sole sources of protein supply. Like other observers they found that "whole" caseinogen acted better than

either of the hydrolyzed products, and that again the ferment digest was superior to the acid one. The ratio between the three as sparers of protein worked out as follows:—

Caseinogen	Caseinogen (Pancreatin)	Caseinogen (Acid)
100	107	127

On the assumption that the work of Abderhalden and others was correct they concluded that the acid hydrolytic products must be accepted as more than protein sparers—that such products, provided they be not too fully hydrolyzed, can take part in actual synthetic processes. Like the majority of workers, they held that in gastro-intestinal digestion there was a complete degradation of the food protein to the simple amino acids or groups of these, and that certain nuclei (probably polypeptide in nature) were absorbed unchanged. It was with the help of these, and probably certain other nitrogen-containing and nitrogen-free groups that the new protein was built up.

Abderhalden [1909, 1] also showed that it was impossible to prevent tissue waste with caseinogen minus tryptophan. Three diets were used (1) fully digested caseinogen, (2) fully digested caseinogen from which the tryptophan had been removed, (3) fully digested caseinogen from which the tryptophan had been removed and then the proper amount again added. Nitrogenous equilibrium was obtained with (1) and (3) but not with (2). In support of these experiments are the further experiments of Abderhalden [1908, 3] in which he showed that nitrogenous equilibrium could be obtained by the products of incomplete acid hydrolysis. He used edestin partially hydrolyzed by treatment for five days at 20° C., with 70 per cent. sulphuric acid; this product consisted of curious polypeptide substances containing glutamic acid, tryptophan and leucine. Similar results were obtained when elastin, hæmoglobin and keratin were treated in like manner. From the extremely interesting experiments of Abderhalden and Ollinger [1908] the nature and constitution of the *whole* digest evidently plays its part. They starved a dog and within seventeen days the weight of the animal fell from 8820 gms. to 7120 gms. Then they gave the animal 3·03 gms. of nitrogen in the form of fully digested caseinogen, but after six days of this diet there was no increase of the animal's weight. The nitrogen intake was increased to 3·99 gms. of the same digest, but still without effect. Then the caseinogen digestion products were replaced by an equal amount (3·99 gms.) of nitrogen from fully digested horse flesh, and after twenty-one days on this diet the weight of the animal rose from 7000 gms. to 8400 gms. That this

was not due to mere storage of unutilized nitrogenous material was clearly demonstrated by the fact that when the animal was starved again the daily loss of nitrogen was quite similar in amount to that which took place in the first instance without any previous protein storage. If this had been a mere accumulation of nitrogenous products in the tissues a great loss of nitrogen during the first days of starvation might have been expected. In all these experiments the diet was made up of a mixture of fat and carbohydrate in addition to the protein digest. Abderhalden, Messner and Windrath [1909], however, in contradistinction to all previous experience, state that they managed to keep an animal in nitrogenous equilibrium with a diet of digestion products and fat *minus* carbohydrates. Of course the explanation here may lie in the fact that the protein was given in such amount that it (or possibly the fat) was partially converted into carbohydrate. And later Abderhalden and Suwa [1910] maintained that they were able to keep a dog in nitrogen equilibrium and to obtain an increase of weight on a protein digestion product alone. Their experiments are, however, very unsatisfactory, as great trouble was experienced in the feeding of the dog with the result that the nitrogen intake was not constant. What is supposed to be the culminating proof that amino acids can serve, apart from peptide groupings, as the sole source of nitrogen in the diet, are the feeding experiments in which artificial mixtures of single purified amino acids were used. Abderhalden and Markwalder [1911] found that the giving of a single amino acid like glycine or alanine with carbohydrate or fat led to a retention of the amino acid in the body, most marked when carbohydrate was used. Abderhalden [1912, 1] carried out a most interesting experiment in which he fed dogs with the following mixture of amino acids: 5 gms. glycine, 10 gms. *d*-alanine, 3 gms. *l*-serine, 2 gms. *l*-cystine, 5 gms. *d*-valine, 10 gms. *l*-leucine, 5 gms. *d*-isoleucine, 5 gms. *l*-aspartic acid, 15 gms. *d*-glutamic acid, 5 gms. *l*-phenylalanine, 5 gms. *l*-tyrosine, 5 gms. *l*-lysine, 5 gms. *d*-arginine, 10 gms. *l*-proline, 5 gms. *l*-histidine and 5 gms. *l*-tryptophan = 100 gms. = 13·87 gms. N. with sugar, etc. He managed in one instance to keep a dog for eight days in nitrogen equilibrium (even slight retention took place) and in another for six days. Mitchell [1916] also tried feeding mice with pure amino acid mixtures and obtained good results even when the mixtures lacked tyrosine and phenylalanine, but the absence of tryptophan was fatal.

Abderhalden, Frank and Schittenhelm [1909] have also carried out digest feeding experiments on a human subject, a boy of twelve, with a stricture of the œsophagus and on whom gastrostomy had been

performed. The experimental material used was flesh completely digested by trypsin and erepsin. The result of feeding per rectum for fifteen days with these digestion products as the main source of protein was that nitrogenous equilibrium was attained and there was even a certain retention of nitrogen. The body weight increased and the general condition was good. Unfortunately the experiment could not be prolonged as towards the end the enemata were not well retained, and this was of course followed by a diminution in absorption.

The Value of Amides and Ammonium Salts.

The nutritive value of amides like asparagine is another question very closely related to that of the maintenance of nitrogenous equilibrium and the growth of animals fed on mixtures of amino acids. It has been very actively discussed by agriculturists, as amides play a large part in the nutrition of the herbivora. It is also of great interest from a general point of view, particularly as regards the question of resynthesis in the body; if it can be proved that an animal can thrive when fed on a single amide or an amide mixture it is indirect evidence in favour of a transmutation of amino acids.

Mercadente [1875] was one of the earliest workers to suggest that the formation of protein could take place from asparagine, particularly in plants. He believed that a decomposition first took place. Schulze [1878] also recognized the possibility of such a synthesis occurring, although he found it difficult to believe that the simple direct union of the amide with the nitrogen-free substance could yield protein. Sachse [1876], on the other hand, thought that asparagine formed protein simply by the addition of fatty aldehydes. O. Loewi [1909] believed that asparagine was converted into protein in the presence of carbohydrate by a series of condensations. O. Loewi [1915] has also maintained that such a synthesis in the vegetable organism from very simple products is possible. He propounds a most interesting scheme of hypothetical changes.

As regards animal metabolism, Zuntz [1891] suggested that in the herbivora before utilization the amide was built up into a protein by the aid of "Pansen" (intestinal) bacteria and that the animal lived on the protein thus formed. Müller [1906] claimed that he had definitely proved that this hypothesis of Zuntz was correct. He found that these bacteria could form, not only from asparagine, but also from ammonium tartrate, higher molecular nitrogenous substances which in part resemble native protein, and in part peptone. The peptone formation represented about 39 per cent. of the asparagine used, and

the native protein about 10 per cent. In the ammonium tartrate experiment after twenty-four hours' incubation about 28 per cent. of the total nitrogen present was in the form of native protein and 67 per cent. in that of peptone. In support of his work he quoted Gerlach and Vogel [1901] who stated that protein-forming bacteria were widely distributed in nature (in the soil) and that if the conditions were suitable these bacteria acted rapidly and well. They found that nitrogen in the form of nitrates was converted quantitatively into insoluble protein in the presence of glucose. Ammonium salts could also undergo the same change, but it proceeded at a slower rate. Müller further carried out a series of feeding experiments with this bacterial protein, which he prepared in sufficient amount, and found that if it were added to the diet of a dog it could entirely replace the ordinary protein supply. Schulze [1908] in a general discussion of this question held that this conversion of the amide into protein was probably correct. He thought the fact that ammonium acetate acted as well as the asparagine strongly supported this contention. He did not come to any final decision as to how the bacteria really brought about the synthesis.

Much other work exists in favour of asparagine acting as a partial substitute for protein, and it would seem that the form in which it is given, plays quite an important part. Thus Lehmann [1906] found that he could obtain a greater protein sparing effect and even a retention of nitrogen, if the asparagine were given embedded in celloidin, in order to cause slow utilization, than if the asparagine were simply given free in the food. Müller [1907] confirmed this work. He found that when the asparagine embedded in the celloidin was given to dogs there was a retention of nitrogen in the body practically equal to twice that found when asparagine was given free. He also stated that if equal amounts of asparagine and serum albumin were given by this celloidin method (the difference in caloric value between the two being made up by means of carbohydrate), they acted equally well in bringing about retention of nitrogen. Kellner [1906], on the other hand, in his experiments was quite unable to find any difference between the action of free asparagine and asparagine embedded in celloidin.

Voltz [1906] and Voltz and Yakuwa [1908] tested the effect on dogs of the addition of different nitrogenous substances, ammonium acetate, acetamide, glycine and asparagine and a mixture of all four, to a basal ration consisting of meat, rice, lard and bones. They found that there was no marked retention of nitrogen after the addition of asparagine and that glycine was more or less indifferent in action.

Acetamide, on the other hand, caused a retention of nitrogen of about 0·2 gm. per diem, and ammonium acetate a retention of 0·4 gm. nitrogen per diem. Munk [1883] denied that asparagine could be even considered a protein sparer in dogs. Mauthner [1891] also tested asparagine as a protein sparer on the dog, but without decisive result. On the whole, he inclined to the belief that there was evidence of a limited sparing action.

As regards the herbivora asparagine is claimed by many workers to be a very efficient protein sparer (Weiske [1894]). Voltz [1907] maintained that amide bodies could replace about two-thirds of the protein in the food of adult ruminants. He concluded that, in all probability, the herbivorous organism could build up its highly complex protein out of a comparatively limited selection of amide bodies. The figures given, if not absolutely convincing, are of great interest. Von Strusiewicz [1906] also showed that sheep could have a very large proportion of the protein in their diet replaced by amide nitrogen. Kellner, Eisenkolbe, Flebbe and Neumann [1910] concluded from their experiments that asparagine and ammonium acetate can replace the protein necessary for maintenance but they cannot be utilized for protein formation even in starving animals. Morgan, Beger, and Westhauser [1910, 1911] stated that if ammonium acetate be added to a diet carbohydrate rich but protein poor it may be utilized for maintenance and even for milk production.

On the other hand, there is much contrary experimental evidence to the retention of nitrogen in animals fed on asparagine as their sole source of nitrogen. Politis [1891] could not obtain any evidence of the protein sparing power of asparagine, when this substance was fed to rats as the sole source of nitrogen, with an otherwise abundant diet and the same result was obtained by Henriques and Hansen [1907]. These workers found, however, that although amide substances obtained from young growing plants could not replace the nitrogen of the food, they could exert, to a limited extent, a protein sparing action.

In a very interesting paper on this question Kellner [1900] pointed out that the protein sparing action of asparagine could only be demonstrated on a protein free or protein poor diet. If it were given with a diet rich in nitrogen there might even be a stimulation of the nitrogen metabolism. He also observed the same sparing action with ammonium acetate when added to a protein poor but carbohydrate rich diet. Lüthje [1906] demonstrated that it was impossible to keep rabbits alive on an abundant carbohydrate diet, when the sole source

of nitrogen was the protein free amide material obtained from fresh potatoes.

A fierce controversy has raged over the question as to whether, ammonium salts can be used as the source of nitrogen in a diet. It is generally admitted that a retention of ammonium salts may take place, but does this retention indicate a synthesis of protein? The work of Knoop and Kertess [1911] and of Embden and Schmitz [1910], as well as the generally accepted fact that glycine can be readily synthetized in the body (see. p. 69), ostensibly demonstrated that the organism could on occasion synthetize amino acids, and greatly stimulated research. The fact that the blood ammonia content may rise, following the giving of urea as shown by G. D. Cathcart [1916] and by Barnett and Addis [1917], amongst others, really places this substance on a par with ammonium salts and they may therefore best be considered together.

The main workers in the field have been Grafe and Abderhalden. Grafe and Schläper [1912] planned their experiments with great care and the conditions under which they were carried out were practically always the same, viz. the animal was prepared by a previous fast of some days, following which there was a pre-period in which the diet was nitrogen free and consisted of abundant carbohydrate and fat. The mean of the last three days of the pre-period and of the first three days of a similar after period served as a measure of the endogenous metabolism, and was used to determine the nitrogen retention in the principal period during which nitrogen was added to the carbohydrate fat diet in the form of an ammonium salt or of urea (Grafe and Turban [1912]). By this method Grafe was able to secure a well-marked reduction of a negative nitrogen balance, and an occasional positive balance on particular days, in the principal period. He claimed that very considerable amounts of nitrogen were retained, e.g. in one experiment [1912, 2] on a pig 14 gms. of ammonium citrate nitrogen were retained in sixteen days. Young growing pigs proved more suitable for these experiments than dogs because they took the food readily and put on weight with astounding rapidity.

Grafe's [1913, 1] most favourable results were obtained in his later experiments by a modification of the procedure; after reducing the nitrogen metabolism to a minimum in the usual way in a further pre-period he gave a small amount of protein equal to from 0·5 to 0·7 of the maintenance minimum; this intake of protein was continued in the principal period during the administration of urea alone or of ammonium citrate and urea. The nitrogen balance remained negative

in the pre-period in spite of the small quantity of protein, but it became definitely positive in the principal period. Grafe points out the importance of making the difference between the nitrogen fed in the form of ammonium salt and urea on the one hand and the protein on the other as great as possible, stating that it should be 3·5 times the value of the maintenance minimum and he criticises the small amount (1·3 times the maintenance minimum) used by Abderhalden and Lampé [1913, 1]. In a further series of experiments, Grafe [1913, 2, 3] found he could not get a positive balance with urea given alone but when given with ammonium citrate, with or without a small amount of protein, a positive balance resulted. He also found [1914] that a certain amount of nitrogen retention occurred when an inorganic salt, ammonium chloride, was substituted for the organic salt, provided that it was given in small or medium doses; in large doses it exerted an injurious influence. As the result of his experiments Grafe [1913, 3] came to the conclusion that there were three possibilities as regards the nitrogen retained: (1) ammonia may be retained as such, (2) it may be fixed, i.e. enter into some form of combination not protein, or (3) it may actually spare body protein by conversion into some protein like body. He decided in favour of the last.

Abderhalden and his co-workers Hirsch and Lampé carried out their experiments on the same general principles as Grafe, but the experiments differed in various details and were not planned with an equal uniformity. The first results were published in 1912 immediately after the appearance of the paper by Grafe and Schläper; they dealt with the effects of superimposing ammonium carbonate or ammonium citrate on an abundant carbohydrate fat diet. Although the nitrogen balance remained negative some of the added nitrogen was undoubtedly retained. Later Abderhalden and Hirsch [1912, 2] published details of two experiments which lasted 103 and 111 days respectively in which ammonium salts were added to a nitrogen free diet. In spite of the difficulties associated with the continued administration of ammonium salts from vomiting and diarrhœa, a retention of some of the ingested ammonia undoubtedly occurred, although the authors, quite rightly, urged caution in the interpretation of the results on account of the variations which were present in the daily nitrogen balance. Abderhalden and Lampé [1912, 2] admit that, although they were unable to obtain such favourable results as those obtained by Grafe, ammonium salts exercised a protein sparing action but, as the nitrogen equilibrium resulting was only transitory, they considered the facts were best explained by the retention of the nitro-

gen in some non-protein form. They doubted [1912, 1] the existence of any marked synthetic capacity on the part of the tissues on the ground that experiments on dogs, in which the nitrogen was supplied in the form of gelatin and ammonia, failed to show a positive nitrogen balance, although there was undoubted retention of nitrogen. They maintained that if the cells could synthetize amino acids a much better result should be gained if the task imposed on them were merely that of synthetizing the few amino acids which were lacking in gelatin, than if they were required to synthetize the whole series present in the protein molecule.

Underhill and Goldschmidt [1913, 1, 2, 3] using a simple one day superimposition method also found that a retention of nitrogen took place after the administration of organic ammonium salts but no such result followed the giving of ammonium chloride. Taylor and Ringer [1913] also examined the problem and found a retention after the giving of ammonium carbonate. They held that the presence of carbohydrate is not essential, as definite retention took place in the case of phloridzinized dogs. Henriques and Andersen [1914, 1] found that a temporary retention of the nitrogen of ammonia salts took place even when these were given intravenously. Caldwell and Clotworthy [1916], on the other hand, were quite unable to find any evidence of retention of nitrogen after the administration of either organic or inorganic ammonium salts.

The whole question, however, of the actual positive retention of nitrogen after the giving of these ammonium salts has been further complicated by the interesting work of Pescheck who found [1911, 1912, 1913, 1914] in his first series of experiments on dogs that there was a slight temporary retention of nitrogen, even in the absence of added glucose, when ammonium acetate was given by the mouth and a definite nitrogen loss when it was injected intravenously. Ammonium tartrate always had a toxic effect but asparagine led to definite retention. Later he showed that sodium acetate, citrate and lactate as well as magnesium citrate could all act as "protein sparers" when added to a diet. He also showed that in the case of sheep, in spite of the diuresis produced, the addition of sodium acetate both with a protein poor and a protein rich diet led to a retention of nitrogen. He inclined to the view that in the case of the ammonium salts and asparagin the protein building capacity of the intestinal bacteria might be the real casual factor, but this cannot account for the results with sodium acetate for which he could offer no explanation. May it not, however, be due to the fact that as Elias and Kolb [1913] have pointed out alkalis have

a definite inhibiting action on glycosuria, hence retention might be indirectly brought about by a better utilization of carbohydrate? Abderhalden [1916] discussed the whole question again and observed that, in addition to the salts already mentioned, the giving of sodium nitrate, although the nitrate could all be recovered again in the urine, led to a definite retention of nitrogen. Abderhalden was quite convinced that although there was actually a diminution in the nitrogen output, none of these salts played any part in the actual nitrogen metabolism. He reached the general conclusion that the nitrogen balance alone neither gives nor can give much information regarding protein metabolism; he quite rightly points out the mere addition of ballast to the food in the form of cellulose may convert a positive balance into a negative one by the increased removal of nitrogen by way of the intestine.

It is quite evident then, that if the body can synthetize amino acids or eventually protein from urea or ammonium salts and the products of the metabolism of carbohydrate or fat it does not readily do so; the results obtained with digests of meat are always incomparably superior.

The Fate of Amino Acids.

So far consideration has been given to feeding experiments with digestion products of proteins—mixtures of amino acids, simple and compound, known and unknown. The point now to be discussed is the fate of the amino acids introduced into the body either singly or in groups, and either by the mouth or parenterally. It would appear that amino acids are utilized when the active form natural to the body is fed or injected, whilst the other form is excreted unchanged. Likewise when a racemic amino acid is given, the natural form is burned and the abnormal one appears for the most part unchanged in the urine. Levene and Meyer [1909], for instance, found after feeding an animal with natural alanine that all the nitrogen appeared in the urine within twenty-four hours as urea, 90 per cent. of it being excreted within nine hours. On the other hand the optical antipode to the natural alanine (l-alanine) was only partially converted into urea (68 per cent.); the rest appeared in the urine as such. Natural l-leucine was more slowly broken down, about 54 per cent. appearing as urea in the first twenty-four hours, the remainder in the following twenty-four hours. Natural l-phenylalanine was all converted into urea, but the operation as in the case of leucine was slow; d-phenylalanine was only converted into urea to the extent of 31 per

cent. Again the nitrogen of aspartic acid was removed to the extent of 86·6 per cent. in twelve hours, while the optical antipode to the natural l-aspartic acid was only excreted in the form of urea to the extent of 31·6 per cent. Arginine nitrogen was found to be excreted as urea to the extent of 97 per cent. Thompson [1905] also found in the case of arginine that the nitrogen was largely excreted as urea. Lewis [1918] has shown that if glycine be injected intravenously at the rate of 0·2 gm. per kilo per hour it can be completely utilized. An increase in urea formation followed. Differences in the fate of injected polypeptides have been noted mainly by Abderhalden and his co-workers. Abderhalden and Bergell [1903], for instance, found that although glycine when injected subcutaneously into the rabbit was burned completely, yet when the dipeptide glycylglycine was injected glycine appeared in the urine, but on the other hand if glycyl-l-tyrosine (Abderhalden and Rona [1905, 2]) were injected combustion was apparently complete, as neither substance was discovered in the urine. Later as the result of both feeding and injecting a dog with the simple amino acids, glycine and alanine, with polypeptides like glycylglycine and diglycylglycine and alanyl-alanine and diketo-piperazines, like glycineanhydride and alanineanhydride, they showed that the breakdown of these substances in the body of this animal was complete, the nitrogen of the different products injected being excreted for the most part as urea. They concluded further that the breakdown of protein in the tissue resembled that which went on in the intestine. Apparently, however, the proteolytic activity of the tissue enzymes was greater than that of the intestinal ferments as polypeptides which were resistant to the action of trypsin, glycyl-glycine and leucylglycine, for example, were broken down to urea (Abderhalden and Babkin [1906]). Another point noted was that the proteolytic tissue enzymes of the dog are apparently much more powerful than those of the rabbit. In support of this observation they quoted Schittenhelm and Katzenstein [1906] who found that if dl-alanine was injected into a dog only a small part of even the l-alanine was excreted in the urine, whereas Wohlgemuth [1905] found when working with the rabbit that only the amino acid forms found in nature were combusted, the abnormal forms being excreted.

Abderhalden and Teruuchi [1906, 1] tested extracts obtained from the tissues, and found that liver extract could break down glycyl-glycine and leucylglycine, both of which are resistant to trypsin. Abderhalden and Hunter [1906] also tested the juices obtained by great pressure from different tissues (liver, muscle, kidney) and found

that they could decompose various dipeptides (dl-leucylglycine, glycyl-dl-alanine and glycylglycine). The decomposition took place asymmetrically, the amino acid *not* found in nature being the one which was not decomposed. Tissue juices were further tested by Abderhalden and Teruuchi [1906, 2] with like result. Dog serum was also shown to be capable of splitting one dipeptide at least, glycyl-l-tyrosine. Leucocytes and renal tissue, however, were shown by Levene and Meyer [1913, 1, 2] to have little or no influence on the breakdown of dl-alanine. Abderhalden and Rona [1906, 2] attempted to discover whether these tissue ferments might not be reversible in action, but they found no synthesis. Abderhalden and Schittenhelm [1907] tested again the question of the fate of racemic simple amino acids in the body. They found that in the dog the abnormal amino acid was excreted in part. Abderhalden, Gigon and London [1907] carried out an excellent experiment on the injection of d-alanine into the jugular vein of a normal dog and of one with an Eck fistula. In each case part of the amino acid was recovered from the blood which flowed from the upper cut end of the jugular vein during the period that the amino acid was being injected into the lower end. More alanine was recovered from the blood of the normal animal than of the one with the Eck fistula (i.e. with the liver cut out of the circulation) and more alanine from the urine of the operated animal than from that of the normal. Even when the alanine was fed to one of the operated dogs the alanine could be isolated from both the blood and the urine. As regards the fate of the diketopiperazines (glycine anhydride and alanine anhydride), neither in dog nor man was there definite evidence of either being attacked, whereas in rabbits the breakdown took place with the subsequent excretion of part of the amino acid in the urine. Abderhalden [1908, 1] thought that the anhydride compound was probably first converted into the dipeptide, then to the simple amino acids. Abderhalden and Walker [1908] showed later that part of the anhydride appears as such in the urine. Among other workers in this field are Stolte [1904], Plant and Reese [1906], Friedmann [1908]. Their results confirm the data previously given.

The Administration of Amino Acids as a Test of Functional Activity.

The fact that the administration of certain amino acids leads to a rise in the output of urea in the urine has been known for many years (Nencki and Schultzen [1872], Salkowski [1880]), but not until the

β-naphthalene sulpho-chloride method was introduced was there any practical means of ascertaining whether part of the administered amino acid was excreted unchanged. With the introduction of this method, which is roughly quantitative, many new observations have been made. It is well to remember that Schlutz [1910] has shown that in the case of infants and children the normal output of amino acids in the urine is higher than in adults. Glaessner [1907], for example, has made practical use of this utilization of amino acids in the body for testing the functional activity (particularly of the liver in his opinion) of the tissues and organs. He determined first the amounts of amino acids which could be dealt with by the normal tissues (liver?), and found that 25 gms. alanine, 25 gms. aspartic acid, 25 gms. glycine, and about 20 gms. leucine could be dealt with, the nitrogen of the amino acids given appearing as urea. He then tested the tissues under different pathological conditions, and obtained the results given in the following table:—

Pathological Condition.	Amino Acid Given.	Dose.	Result.
Fatty liver.	Aspartic acid.	20 gms.	Marked rise in output of amino acid in the urine.
Syphilitic ,,	Alanine.	20 gms.	Marked rise in output of amino acid in the urine.
Cirrhotic ,,	Glycine.	25 gms.	Rise in output of amino acid equal to about 70 per cent. of the glycine taken.
,, ,,	,,	25 gms.	Rise equal to about 51 per cent. of the glycine taken.
,, ,,	Aspartic acid.	25 gms.	Rise in output equal to about 40 per cent. of the aspartic acid taken.
,, ,,	,,	25 gms.	About 87 per cent. of the aspartic acid taken excreted.
,, ,,	,,	25 gms.	About 90 per cent. of the aspartic acid taken excreted.
,, ,,	Alanine.	20 gms.	About 51 per cent. of the alanine taken excreted.
Phosphorus Poisoning.	Glycine.	20 gms.	Very marked rise in the output of amino acid in the urine.

Bergell and Blumenthal [1904] have also demonstrated that, under pathological conditions, an amino acid, tyrosine, which was not excreted as such after subcutaneous injection into the normal animal, appeared unchanged in the urine if the injections were made after removal of the pancreas. The introduction of the excellent methods of Sörensen and of van Slyke have led to a marked increase in the number of observations on the excretion of amino acids. Masuda [1911], for instance, using the Sörensen method found that in hepatic disease the output of amino acids following their administration (alanine and

glycine) in 5-gram doses was about 20 per cent. greater than in other diseases.

Mode of Catabolism of the Amino Acids.

In the present monograph it is not intended to discuss the course of the catabolism of the amino acids from the purely chemical standpoint, as this is dealt with in another volume in this series. It may be said that after the removal of the amino group by the deaminizing ferments (see p. 56) the fate of the nitrogen free residue resolves itself into the fate of an ordinary fatty acid or an aldehyde. Within recent years there has been much discussion concerning the chemical processes which are involved in deaminization. Among the most active workers in this field are O. Neubauer and his pupils. Neubauer [1908] published an excellent paper dealing with the problem and he pointed out that there were three possible lines of breakdown: (1) oxydative deaminization with the formation of a keto-acid, (2) hydrolytic deaminization with the formation of an oxy-acid, and (3) reductive deaminization with the formation of a fatty acid containing the same number of carbon atoms.

But Dakin and Dudley [1913, 1], Neuberg, and others, have produced evidence which would show that in all probability the breakdown of the amino acid in the body is very similar to that of carbohydrate. They have given the following scheme:—

$$R.CH.NH_2.COOH \rightarrow R.CO.CHO + NH_3$$
$$R.CH.OH.COOH \rightarrow R.CO.CHO + H_2O$$

and therefore possibly

$$\begin{array}{ccccc} & & \text{Glycogen} & & \\ & & \uparrow\downarrow & & \\ & & \text{Glucose} & & \\ & & (C_6H_{12}O_6) & & \\ & & \uparrow\downarrow & & \\ & & \text{Glyceric Aldehyde} & & \\ & & (C_3H_6O_3) & & \\ & & \uparrow\downarrow & & \\ \text{Lactic Acid} & \leftarrow & \text{Methyl Glyoxal} & \rightarrow & \text{Alanine} \\ (CH_3.CHOH.COOH) & \rightarrow & (CH_3.CO.CHO) & \leftarrow & (CH_3.CHNH_2.COOH). \end{array}$$

Sugar Formation from Amino Acids.

One problem connected with the disposal of the non-nitrogenous rest which is of considerable interest may be briefly referred to here, namely, the production of carbohydrate from this rest. Although it is generally supposed that the carbohydrate of the food is mainly concerned with the energy needs of the organism it is gradually becoming apparent that carbohydrate is closely concerned with other tissue requirements. Lusk [1910] has carried out much important work on

this subject. He has demonstrated that glycine and alanine are converted into glucose and that three of the carbon atoms contained in aspartic and glutamic acids are also so converted. As regards the sugar production from meat he has found that fifty-eight parts of glucose may be formed from one hundred parts of meat, and he has further calculated that 45 per cent. of the total sugar production from protein in diabetes may arise from the four amino acids glycine, alanine, aspartic and glutamic acids. Among other workers who have investigated this problem are Embden and his pupils [1904, 1905], Glaessner and Pick [1907], Halsey [1904], Röhmer [1910], and Ringer and Lusk [1910]. Janney [1915, 1] found that the sugar yielding power varied with the protein used. He with Blatherwick [1915] also found that when protein from human muscle was tested there was a formation of sugar at least up to 50 per cent.

CHAPTER IV.

DEAMINIZATION.

General.

THE question of deaminization of the protein molecule must now be dealt with. Evidence has been gradually accumulating which points to the deaminizing capacity of the tissues, as being one of the fundamentally important activities. It has long been known that shortly after the ingestion of protein there follows a marked rise in the output of nitrogen in the urine for the most part in the form of urea (see p. 90). The cause of this rise was long a matter of speculation: did this nitrogen come from the protein ingested, or did it come from the tissues? The work of Nencki and his school helped to show with some degree of certainty the important part played by the ingested protein digestion products, but it was not until modern experimental methods were introduced that more or less direct evidence was obtained in favour of the view that the urea could come direct from the ammonia liberated by the deaminizing activities of certain of the tissues. To meet the objection that the energy loss would be considerable if an extensive deaminization took place, direct estimations of the energy value of certain amino acids and of the products resulting from deaminization have been carried out, and it has been shown that no material loss occurs. What most workers lost sight of in considering this question is the fact that the actual demand for nitrogen by the body is not high, and that the fundamental use of nitrogenous food-stuffs is to repair protein tissue waste and not to supply energy in the form of a compound of a fatty acid and ammonia. Protein is of most importance to the tissues not because of any inherent virtue in itself, but merely because it contains within its molecule certain compounds of nitrogen more or less ready for building purposes.

Lower forms of life can build up the amino acids for their protein formation from ammonium salts or nitrates present in their nutritive mixtures, although they utilize the amino acids and flourish amazingly on them if given the opportunity. Abderhalden and Rona [1905, 3]

found that *Aspergillus Niger* on a potassium nitrate medium could form its protein which contained glycine, alanine, leucine, glutamic and aspartic acids. Plants work on the same principle, but are even more adaptable, as certain members can utilize the atmospheric nitrogen when required (cf. Lipman [1911]), a power which animals never possess, as Krogh [1906] and Oppenheimer [1907] have so clearly shown.

This deaminizing activity is fundamental in its nature, probably it is essential for synthetic activity. The idea that such a splitting of the protein molecule into a nitrogenous part and a non-nitrogenous part takes place is by no means a new one. Voit many years ago accounted for the comparatively rapid output of nitrogen and the comparative slow output of carbon after a protein meal on the grounds that soon after absorption there was a splitting of the protein molecule into a nitrogen rich part which was rapidly dealt with, and a nitrogen poor part which was more slowly utilized.

The Presence of Ammonia in the Portal Blood.

Great stress was formerly laid on the fact that the content of the portal blood in ammonia was higher than that of the systemic blood. It was believed that these observations definitely decided the point in favour of practically an immediate deaminization. Thus Nencki [1896] and his pupils, when experimenting with dogs on which the Eck fistula operation had been carried out, stated that the portal blood contained three to four times more ammonia than the normal systemic blood although the absolute amounts in both instances were small. Further that the ammonia content of the systemic blood approximated to that of the portal after an Eck's fistula was made, i.e. the liver, cut out of the circulation, no longer acted as a check to the entry of ammonia into the systemic blood. They also noted that the gastric and intestinal mucous membrane contained more ammonia at the height of digestion than when at rest. Horodynski, Salaskin and Zaleski [1902] confirmed this work, and found that, although there was a definite increase of ammonia in the portal blood compared with the systemic blood during digestion, even during starvation the portal blood contained more ammonia than the systemic. They also noted that the ammonia content of the tissues and organs (the intestinal mucous membrane, liver, etc.) intimately connected with the absorption and utilization of food was diminished during starvation. Biedl and Winterberg [1902] denied that the portal blood contained, as a rule, more ammonia than the systemic, although they admitted

that the portal blood contained at times more than the average amount of ammonia. Folin and Denis [1912, 2] maintained, however, that their experiments conclusively demonstrated that the increased content of the portal blood in ammonia was due not to deaminization changes in the small intestine but to absorption of ammonia from the large intestine. Cohnheim [1909] gave almost direct evidence of the possibility of deaminization taking place during absorption. He found that if isolated fish intestine, into which he had placed proteose solution, were floated in Ringer's solution, partial deaminization took place, as shown by the fact that ammonia and an unknown base appeared in the Ringer solution. In conjunction with Makita [1909] he repeated this experiment introducing into the intestine, glycine and tyrosine as test substances. Glycine yielded ammonia, or perhaps more correctly a volatile base, and from tyrosine ammonia was similarly obtained. Similar experiments were tried with the intestines of dogs and cats, but without much success. Cohnheim [1912] in a later series of experiments, already referred to (p. 25), showed that after the introduction of amino acids into loops of fish intestine even up to 70 per cent. of the nitrogen, which passed out into the Ringer solution in which the intestine was suspended, was in the form of ammonia.

Deaminizing Capacity of Tissues.

(a) *In vitro.*

As regards the presence of enzymes in the tissues which led to the formation of ammonia, the work of Loewi [1898] and of Jacoby [1900] was the earliest, although their evidence was not very complete. Jacoby found that in the fluid which he obtained by pressure from pulped liver tissue the amount of ammonia increased after incubation at 40° C. This ammonia, he believed, was derived from substances like amino acids which were not deaminized by boiling with acids. Loewi [1898] showed that the amino group in glycine was converted by the action of liver pulp into a substance which, although it was not urea, resembled this substance, particularly as regards the ease with which it could give up its ammonia. Lang's paper [1904] was the first serious attempt to attack the question of deaminization by testing the action of the different tissues on the amino acids themselves. Lang obtained results which showed that the deaminization in the tissues was extremely active, although there was a certain degree of specificity, i.e. some amino acids were quite untouched by one tissue, whilst actively broken down by another. Thus glycine was much more

readily broken down by the intestine than the liver, and it was not attacked by the spleen tissue at all. He found, too, that the amides, asparagine and glutamine, gave up their ammonia very readily in the presence of any tissue. The short experiments which he carried out with fresh tissue under aseptic conditions gave better results than the long experiments, in which he used material preserved by means of an antiseptic.

Miss Bostock [1911] reinvestigated the question, and repeated some of Lang's experiments. She found that Lang's main contention was true that deaminization took place, but she also found that the degree of deaminization was much less marked than Lang described and that his results with tissue pulp with and without antiseptic could not be fully substantiated. Like Lang, she showed that the amide bodies yielded their nitrogen with greater readiness than the amino acids. Of course, on account of the well-known capacity of many bacteria to deaminize, one of the objections which has been raised against the so-called "aseptic" autolytic experiments is that they are not really aseptic and that the breakdown observed is due to bacteria. Miss Bostock found that it was almost impossible to get a real aseptic test. She demonstrated, however, that although part of the ammonia given off might be due to the bacterial action, additional deaminization which might be ascribed to ferments present could be proved to take place. Incidentally it was found that the deaminizing activity started at a very early age in fœtal life. Levene and Meyer [1913, 1] found that leucocytes and kidney tissue had little or no action on dl-alanine or [1913, 2] on glycine, aspartic acid, leucine and asparagine. Schweizer [1917] questions the existence of a deaminase. He found that an oxidase like tyrosinase can split off ammonia readily from an amino acid like glycine. Asparagine can also be readily deaminized but not leucine. This result may be explained by the experiments of Folmer [1917] who claimed that his work demonstrated that the so-called oxidase-tyrosinase is in reality a mixture of two ferments, a de-aminase and a phenolase.

(*b*) *In vivo.*

The results obtained by *in vitro* experiments do not always tally with those obtained *in vivo.* Lang's and Miss Bostock's observations for example were at one in showing that after digestion with the different organ pulps an amide like asparagine gives up its nitrogen with greater ease than an amino acid like glycine. Levene and Kober [1908], amongst others, had found that if glycine were given *per os*,

practically the whole of its nitrogen appeared rapidly in the urine as urea, whereas in the case of asparagine there was a definite retention of part of the nitrogen. Miss Bostock also carried out a few experiments in connexion with this retention, and she confirmed the observation of Levene and Kober. She found that within eight hours practically all the nitrogen of glycine when given *per os*, appeared in the urine as urea, but in the case of asparagine only about 63 per cent. of the nitrogen reappeared in the same time as urea in the urine. Apparently then in the digest conditions *in vitro*, the amino group attached to the carboxyl group of an amide, is much more readily split off than the amino group in the *a* position of an amino acid, whereas *in vivo* a certain protection is given to the amide which is not extended to the amino acid, or else there is an actual retention of the nitrogen of asparagine for synthetic purposes (see p. 44).

At any rate this evidence, slight as it is, suffices to show that the method of research instituted by Lang for the investigation of the deaminizing capacity of tissues is by no means representative of the changes which go on within the normal living tissues.

Other evidence in support of deaminization being a normal intravital change has come from the many experiments which have already been given in detail (p. 50) carried out on the feeding of animals with different amino acids. Thompson [1905] found in the case of arginine that the nitrogen was largely excreted as urea; the amount excreted, however, differed to some extent in the different animals, and apparently with different diets. He noted that one part of the ingested nitrogen was excreted as urea soon after ingestion but that another part seemed to be metabolized more slowly. He believed the latter—the slow excretion—came from the deaminization of the ornithine moiety of the arginine molecule. He further noted that if the arginine were injected subcutaneously a much greater proportion of its nitrogen was excreted as urea. Neuberg and Langstein [1903] also showed that after the administration of large doses of alanine to rabbits, lactic acid could be recovered from the urine. The appearance of the oxy acid, lactic acid, is contrary to what one would have expected from the work of Neubauer, who concluded that pyruvic acid, a keto acid, was formed on deaminization of alanine. In experiments which exclude any possible bacterial action, Mayer [1904] has shown that after the subcutaneous injection of diamino-propionic acid there was a small output of glyceric acid (a di-oxy acid). The formation of homogentisic acid from tyrosine and phenylalanine in cases of alkaptonuria is direct evidence that deaminization can take place readily. Bang [1916, 2] showed that the

increase in the amino acid content of the blood was not great after the giving either a large or small dose of glycine or alanine; no rise followed the giving of leucine although the blood urea content increased. He also found that the ingestion of proteins rich in glycine led to a more marked increase of the rest nitrogen of the blood than the ingestion of those poor in glycine.

G. D. Cathcart [1916] who investigated the whole question as to the effect of the intravenous injection of ammonia, urea and glycocoll on the nitrogen distribution in the tissues, clearly showed that although there was no evidence of the synthesis of any of these substances into protein there was abundant proof of their further metabolism, particularly in the liver. Ammonia was converted into amide nitrogen both in liver and muscle, urea was curiously enough partially deaminized in the liver and muscle and glycine was deaminized in the liver and partially converted into amide both in liver and muscle.

Examples of this widespread deaminization, in reality the preliminary step, in the great majority of instances, to further metabolism, could be indefinitely extended. In the ordinary course of the metabolism of nucleo-proteins this deaminization plays a most important part.

One point may be briefly touched upon here, namely, the part played by the tissues in bringing about the breakdown of the nitrogen grouping. After the classical work of Schröder, and others, it had always been more or less implicitly assumed that the liver played the most important, if not the sole part, in disposing of the "waste" nitrogen in the form of urea. Although much reliable evidence exists to support the view that the liver can deal with amino acids as well as ammonia, such as that of Jansen [1915], who found a definite rise in the urea of the blood after perfusion of the liver with glycine, alanine, etc., and Löffler [1916] who also found a similar increase after glycine, leucine, serine, etc., but none after perfusion with tyrosine, cystine, and taurine, attacks have been made on the position. Thus Matthews and Miller [1913] found that, even when the liver had been cut out of the circulation for fifteen months, some 80 per cent. of the total nitrogen excreted was in the form of urea. Later, Matthews and Nelson [1914] showed that if amino acids were injected intramuscularly there was a subsequent increase of the urea and ammonia in the urine although Hoagland and Mansfield [1917] held that as the result of their experiments the muscle played but a small part in urea formation.

Fiske and others [1913, 1914] maintained that perfusion of the liver with amino acids did not give rise to urea formation, that the rise

in the urea content of the blood after intravenous injections of amino acids was as great without the liver being in circulation as with it functioning normally. Taylor and Lewis [1915, 2] came to the general conclusion that urea formation was a function common to all cells.

Deaminization in the Lower Forms of Life.

If we turn now to the lower forms of life we find that deaminization is a normal function of both moulds and the micro-organisms of putrefaction. Shibata [1904] made a dry acetone preparation of *Aspergillus Niger* and tested its deaminizing activity on various substances. It split off ammonia from urea, biuret, acetamide, asparagine and alanine but did not decompose guanidine, allantoin and uric acid. In the case of asparagine the yield of ammonia was small, and he concluded on quite insufficient evidence that in this case the ammonia came from the amino acid NH_2 group and not from the amide NH_2 group. The deaminizing capabilities of bacteria have been investigated by Brasch and Neuberg [1908], Neuberg and others [1909], Brasch [1909], Borchardt [1909], whilst the action of yeast has been studied by Ehrlich [1909]. Weinland [1906], in an extremely interesting paper on the excretion of ammonia by fly larvæ, gave excellent evidence in support of deaminization. The Calliphora larvæ are flesh eaters, and Weinland found that in the course of larval development, when much flesh was eaten, ammonia was given off in large amount.

From all the evidence cited we may conclude that the process of deaminization is a fundamental one in the metabolism of proteins, and particularly of that portion of the protein which serves for dynamic purposes.

CHAPTER V.

INFLUENCE OF THE FOOD ON THE COMPOSITION OF THE TISSUES.

THE disposal of the protein, both in its natural and in the abiuret condition has been dealt with. The question now arises, if the food be of a highly specific nature will it influence in any way the composition of the protein of the tissues? In coming to any definite conclusion on the influence of material ingested, enterally or parenterally, great care must always be exercised in the interpretation of the tissue analyses data. As G. D. Cathcart [1916] has, for example, clearly shown the analysis of perfectly normal tissue may vary within quite wide limits and have no definite relation to diet. The percentage of moisture present for instance varied within 12 per cent.

Evidence from Feeding Experiments.

Abderhalden and Samuely [1905] were the first to offer direct evidence on this question. They attempted to influence the composition of the serum protein by feeding a horse with gliadin which contained 36·5 per cent. of glutamic acid, whereas the serum globulin of the horse contained only 8·5 per cent. and the serum albumin 7·7 per cent. The tyrosine content of gliadin is 2·37 per cent. In their experiments they tested the tyrosine and glutamic acid content of (1) normal serum protein, (2) serum protein after starvation, and (3) serum protein after feeding with gliadin. The horse was first thoroughly bled, it then fasted for eight days, and was subsequently fed with gliadin, which was given in large quantities, for two days (Experiment I.). After a period of rest it fasted again for six days (Experiment II.). Blood was removed from the horse in Experiment I. both during and after the gliadin feeding, but only after feeding in Experiment II. The tyrosine and glutamic acid content of the serum protein removed at the different periods is given in the following table:—

	Experiment I.				Experiment II.		
	Percentage of Substance Present in the Blood Removed During—						
	Before Period.	Hunger Period.	Gliadin Feeding Period.	After Period.	Before Period.	Hunger Period.	After Period.
Tyrosine . .	2·43	2·60	2·24	2·52	2·50	2·55	2·48
Glutamic Acid .	8·85	8·20	7·88	8·25	9·52	8·52	8·00

It will be noted that, under the above conditions, the food had no influence on the composition of the proteins of the serum. The authors held that the necessary change in the ingested protein took place in the intestinal wall, the extra glutamic acid having been split off and probably absorbed separately. Abderhalden, Funk and London [1907] again investigated this question. In this series of experiments they used dogs with Eck's fistulæ and fed the animals with food of known composition (of known glutamic acid content). The blood was examined before and after the special feeding. The foods used were flesh, egg albumin and gliadin. As the result of their experiments they concluded that no food protein was to be found in the blood and that no influence of the constitution of the food on the constitution of the serum protein, more especially as regards the content in glutamic acid was discoverable. They also examined the nature of the protein in the blood cells but found that it too was unchanged. Then Abderhalden, Gigon and Strauss [1907] showed that although the animals were fed on widely differing foods, the tissues of cats, rabbits and hens were practically identical in constitution, i.e. with about 3·3 per cent. of glycine and over 13 per cent. of glutamic acid. Orgelmeister [1906] has also shown that the arginine content of tissues could not be altered by feeding the animal on foods rich in arginine. Nor was he able to alter the arginine content of the tissues by the administration of substances like benzoic acid which might have been expected to combine with the arginine.

Gitkins [1904] studied the composition of the blood during hunger, and found, like Burckhardt [1883], Lewinski [1903] and others, a distinct increase of the globulin fraction of the serum. He also noted that if bread were used in the subsequent feeding of the animal the blood more rapidly returned to its normal composition than when a meat diet was given. He suggested that the albumin of the serum came from the food, and the globulin from the tissues. Robertson [1912] states that in the rabbit, ox and horse, starvation leads to an

increase in the proportion of albumin in serum whereas in the rat and dog the increase is in the globulin fraction. Kerr, Hurwitz and Whipple [1918, 1, 2, 3] found on the other hand that starvation had but little influence on the protein content of serum. They also came to the general conclusion that serum protein was not an intermediate form between food and tissue protein.

Composition of the Tissues.

Pflüger and the majority of workers in this field held that the composition of the tissues did not appreciably alter with changes in the nature and the composition of the food, although as Thomas [1911], and others, have shown the tissues of young animals contain both more water and ash than those of the adult. Rubner, on the other hand, maintained that the composition of the tissues could be altered if they were deprived of certain food materials. Stockhausen [1909] carried out elaborate analyses of the tissues of two sets of dogs. An adult and a young growing dog were fed on a protein (flesh) diet and a similar pair were fed on a diet of rice. He found that the muscles of both the flesh-fed dogs contained more nitrogenous material than the rice-fed; those of the adult dog fed on rice contained 88 per cent. of nitrogen, on flesh 94 per cent.; those of the young dog fed on rice contained 93 per cent. nitrogen, on flesh 94 per cent. A like condition was also noted in the case of the liver. The liver of the adult dog fed on rice contained 65 per cent. of nitrogenous material and that on flesh 88 per cent.; in the case of the young growing dog when rice-fed the liver had 66 per cent. and when flesh-fed 91 per cent. of nitrogenous material. Stockhausen further made some interesting observations on the relation of nitrogen to carbon in the tissues of his rice- and flesh-fed dogs. He found that in the case of the rice-fed dogs, taking both together, the ratio of nitrogen to carbon was as 1 : 3·30 and that in the flesh-fed dogs it was as 1 : 3·28. Therefore even in young growing dogs, when the tissue growth and exchange was extremely active, a very decided change in diet caused but little or no alteration in the composition of the tissues.

Müller [1907] carried out a very interesting experiment in this connexion. He fed a dog for six weeks on a meat-free diet of rice, lard, salts and water, and at the end of this period of feeding he amputated a hind limb the muscles of which were used for an examination of the composition of the tissues. After recovery from the operation he fed the animal for another six weeks on large quantities of horse flesh, and at the end of the period the animal was killed and

muscle was taken from the other hind leg for examination. As a result of his analysis Müller came to the conclusion that a special nitrogenous store material was formed which differed in its elementary composition from muscle protein, as it was particularly rich in nitrogen. He believed that the formation of this nitrogen-rich carbon-poor store substance accounted for the large retention of nitrogen which has been observed from time to time after a diet rich in protein. Diesselhorst [1911] repeated this work of Müller employing the same technique. He used two dogs fed as before on rice and then on meat and found in both that there was a definite gain in the nitrogen content of muscle as the result of the feeding with meat. There was an equally definite gain of carbon so that in the end as the result of his experiments he found that the N : C ratio was practically identical with both diets. Grund [1910] also found a definite alteration in the composition of liver, kidney and muscle tissue as the result of feeding. Orgler [1910] carried out a series of interesting experiments on two sets of puppies of the same litter, one lot fed naturally on the mother's milk, the other by hand with cow's milk. In spite of the fact that growth was taking place and that the dog's milk is richer in protein, fat and ash than cow's milk there was little or no difference to be detected between the chemical composition of the two sets of animals.

Zisterer [1910] has also investigated this question. In the first place he compared the composition of various proteins, gliadin, glutenin, and caseinogen, with muscle protein (syntonin) and has compiled the following table of the amino acid (partial) content in 100 gms. of protein :—

	Syntonin.	Caseinogen.	Gliadin.	Glutenin.
Alanine	4·0	0·9	2·7	0·3
Leucine	7·8	10·5	6·0	4·1
Glutamic acid . .	13·6	11·0	31·5	24·0
Tyrosine . . .	2·2	4·5	2·4	1·9

He argues that if these different proteins, after decomposition in the intestine and absorption were utilized for the formation of the muscle, then a variation in the constitution of the muscle protein ought to be found. In order to test this point small quantities of these different proteins close to the minimal protein requirement were given but no very marked differences were found in the minimum amount required to get the dog into nitrogenous equilibrium. This result did not correspond with what was expected from an interesting calculation

which he gave at the end of his paper as regards the relationship in amino acid content of the different proteins used. He found that four to five times as much caseinogen and two and a half times as much aleuronat must be given in order to yield the amount of alanine necessary to form syntonin, and so on for the other amino acids. This calculated result is extremely interesting in the light of the actual result. It suggests that a certain transmutation of amino acids can and does take place (see p. 69).

One of the deductions to be drawn from the work of Folin and van Slyke is that the tissues have apparently no selective action with regard to the various forms of nitrogen carried to them by the blood stream but that they take up amounts of amino acid, urea and ammonia which depend chiefly on the concentration of these substances in the blood, the higher the concentration the greater the probability of fixation on the part of the tissues. Further, as these three forms of nitrogen are so readily washed out of the tissues, it would be almost unreasonable to assume that they enter into real chemical union with any of the cell constituents. The property of adsorption must, however, always be borne in mind.

Growth of Moulds.

Some interesting work has also been carried out on the influence of the nutritive medium on the growth and composition of moulds. Abderhalden and Rona [1905, 3] for example, found that there was no apparent alteration in the composition of the protein obtainable from *Aspergillus niger*, when it was grown upon culture solutions in which the nitrogen present was in three different forms: (1) potassium nitrate, (2) glycine, and (3) glutamic acid. Ehrlich [1911] has also shown that normal protein can be formed from tyrosine as the source of nitrogen. Even, according to Lipman [1911], atmospheric nitrogen can be utilized by certain yeasts and moulds.

Variation in the Composition of Protein.

Another question of fundamental importance in discussing the minimum requirement of protein, more especially in the light of Michaud's [1909] work, is whether any marked difference can be detected in the constitution of proteins, obtained from different sources in the animal and vegetable kingdom. Osborne and Jones [1909] have carried out some extremely valuable work along this line. They examined the muscle obtained from chicks [1908], from fish [1909], and from the

scallop [1909]. When the various muscles were compared very marked differences in their amino acid content were found. They also showed that the constitution of syntonin was very different from that of muscle itself. They obtained a smaller yield of leucine, proline, phenylalanine, aspartic and glutamic acids and lysine from the former. They came to the general conclusion that glycine, alanine, valine, leucine and proline increase in proportion as we go from the lower to the higher forms of life. The table below from their paper demonstrates their conclusions very clearly.

Substance.	Scallop.	Fish.	Chicken.	Ox.	Syntonin Ox.
Glycine . . .	0·00	0·00	0·68	2·06	0·50
Alanine . . .	+ ?	+ ?	2·28	3·72	4·00
Valine . . .	+ ?	0·79	+ ?	0·81	0·90
Leucine . . .	8·78	10·33	11·19	11·65	7·80
Proline . . .	2·28	3·17	4·74	5·82	3·30
Phenylalanine . .	4·90	3·04	3·53	3·15	2·50
Aspartic acid . .	3·47	2·73	3·21	4·51	0·50
Glutamic acid . .	14·88	10·13	16·48	15·49	13·60
Serine . . .	?	?	?	?	?
Tyrosine . . .	1·95	2·39	2·16	2·20	2·20
Arginine . . .	7·38	6·34	6·50	7·47	5·06
Histidine . . .	2·02	2·55	2·47	1·76	2·66
Lysine . . .	5·77	7·45	7·24	7·59	3·26
Ammonia . . .	1·08	1·33	1·67	1·07	0·83
Tryptophan . .	Present	Present	Present	Present	?
Total . . .	52·51	50·25	62·15	67·30	47·11

These workers [1909] have also found that the different vegetable proteins differ markedly in composition. The proteins of the leguminous seeds most nearly approximate in composition to muscle protein. They suggest that this is probably the explanation why these seeds have proved to be such valuable food-stuffs.

But it must not be forgotten that although we may get much information about the gross composition of the protein molecule as regards its amino acid content this does not give us full information. Abderhalden and Langstein [1910] for example claim to have shown that the composition of the casein obtained from human and cow milk is identical, but Dudley and Woodman [1915] have brought forward evidence, by the racemization method, which demonstrates that the intramolecular arrangements of the amino acids in the casein of sheep and cow milk is not identical, and Dakin and Dale [1919] have shown the same thing for the albumin of the egg of the hen and the duck.

Variations during Development.

Abderhalden and Kempe [1907] carried out some experiments on the composition of the egg at different periods of incubation. They found that tyrosine was the only amino acid which altered in amount and that this alteration was very small: 100 gms. dried fresh egg contained 1·82 gm. tyrosine; after ten days' incubation at 40° there was 2·11 per cent. of tyrosine and after twenty days 2·25 per cent (in these analyses the embryo was also included). The amount of glycine and glutamic acid, the other two substances estimated, hardly varied during the incubation. They concluded that there was no new formation of amino acids during development. Burns [1916] has shown, however, that in the developing chick embryo there is a regular increase in the content of guanidine till the twelfth day of incubation, followed by a marked decrease and then finally by a slight rise.

Transmutation of Amino Acids.

Another important question, in this connexion, is whether the organism has the power of converting one amino acid into another, i.e. can transmutation take place?

Osborne and Mendel [1912], as the result of their long series of feeding experiments with a wide variety of proteins, have come to the general conclusion that in the past the possibility of the transmutation or synthesis of amino acids in the organism has been greatly underestimated. In this connexion the work of Zuntz, and others, on intestinal bacteria already referred to (p. 44) must not be overlooked. See also the work of Thomas [1909].

This question has been investigated by Magnus-Levy [1907], and others, by the study of the ratio of the amount of glycine nitrogen to total nitrogen, the output of hippuric acid being used for the estimation of the glycine. With the exception of gelatin the body proteins contain on an average 4 per cent. of glycine.[1] Now as protein contains some 16 per cent. of nitrogen, and glycine 18·7 per cent. there is present in 100 gms. protein-nitrogen rather less than 5 per cent. glycine-nitrogen. As Magnus-Levy points out if in the benzoic acid experiments a higher value for the quotient (glycine-nitrogen : total nitrogen) be found than this figure (5 per cent.), then there is evidence of the production of glycine from a source other than performed glycine. Magnus-Levy found that the quotient was larger, therefore a synthesis

[1] This value is probably less than the true value, for according to Osborne and others the esterification method by which the amino acid content of tissues has been estimated gives too low results. (See Dr. Plimmer's monographs in this series.)

of glycine had taken place somewhere in the tissues. He has also pointed out as further evidence in favour of such a synthesis the fact that the protein food of a suckling animal is remarkably poor in preformed glycine—he estimated that milk proteins, at the outside, contain only from ·1 to ·3 per cent. of glycine, and yet it is found that from 100 gms. of this protein a suckling calf can in a short period build up 78 gms. of tissue protein containing about 2·5 gms. of glycine. It is suggested that in the formation of this glycine two courses are open either (1) the *in vivo* breakdown of protein is identical with that *in vitro*, and that the other amino acids formed are converted by oxidative processes into glycine, or (2) that the *in vivo* breakdown of protein is not the same as that observed *in vitro*, in that a greater amount of glycine is formed. Magnus-Levy rather inclines to the first hypothesis. As an example of his experiments the following may be cited. A rabbit weighing 1500 gms., containing thus some 200 gms. protein with about 6·6 gms. of glycine, excreted after treatment with benzoic acid 8 gms. of glycine. During the course of the experiment the weight of the animal fell to about 1250 gms.; a loss of 250 gms. which (even supposing the total loss were due to protein utilized) would have only yielded a little over 1 gm. of glycine. Magnus-Levy later suggested that the glycine which was excreted in the previous experiments might have been formed from other amino acids, such as leucine and alanine, which had combined with the benzoic acid, the resulting product having been then converted by a process of oxidation into hippuric acid. He carried out a series of experiments to test this hypothesis, but did not obtain positive results. He tested some ten preparations but in none did he get conversion into hippuric acid.

Abderhalden and Funk [1909], and Abderhalden and Hirsch [1912, 1], also investigated this question, and came to the conclusion that the only amino acid of which definite proof of formation or synthesis *in vitro* can be obtained is glycine. They also utilized the evidence obtained by feeding with caseinogen in which there is no glycine; the animal was rapidly got into a state of nitrogenous equilibrium. It must therefore be concluded that synthesis of glycine can be readily brought about with little or no strain on the protein anabolic processes. They were unable to confirm an observation of Henriques [1909] regarding the synthesis of lysine in the animal body (rats). Henriques stated that although zein, which lacked glycine, lysine and tryptophan, could not maintain life when given in the food as the sole source of nitrogen, yet under the same conditions this could be successfully accomplished by gliadin, which lacked lysine. The deduction was that

either lysine was not required or that it could be synthetized from some of the other amino acids present. Abderhalden and Funk repeated this experiment on a dog and found that nitrogenous equilibrium could be obtained, but their analyses showed the presence of a small amount of lysine in gliadin.

Wiechowski [1906] also found that there could be a greater output of glycine after dosing a rabbit with benzoic acid than could be accounted for by the amount of glycine present in the tissue proteins. He came to the extraordinary conclusion that in the rabbit glycine was the principal source of the urea, and that all the other amino acids were converted into glycine before their final destruction in the body. Such a conception does not seem necessary to explain the facts already known as regards the fate of the amino acids in the body. Ringer [1911] also found that goats and rabbits can excrete more glycine (as hippuric acid) than is found preformed in the tissues.

On the other hand Brugsch [1907] stated that the amount of glycine which was excreted after giving benzoic acid was equal in amount to that which existed as such in the tissues and which could be obtained by hydrolysis *in vitro*. Tsuchiga [1909] obtained results which varied considerably and no definite conclusion could be reached. He maintained that according to the dose and the method of administration of the benzoic acid, the amount of glycine excreted as hippuric acid fluctuated greatly. Yosikawa [1910] made the interesting observation that whatever may be the position in the mammal the avian organism cannot synthetize hippuric acid even when glycine is fed at the same time as benzoic acid.

The evidence then is in favour of a synthesis of glycine taking place in the tissues, and this change in all probability is brought about by the decomposition of some other amino acid with a longer carbon chain. Epstein and Bookman [1911] are quite convinced that glycine can be synthetized in the body but they could come to no decision as to whether it arose from the breakdown of higher amino acids or by synthesis from simpler products. In support of the first hypothesis they [1912] have shown that the administration of benzoyl leucine, although not of leucine itself, with benzoic acid yields hippuric acid but the administration of alanine free or as a benzoyl compound gave a negative result [1914]. Abderhalden and Strauss [1914] on the other hand found that a slight but definite increase in the output of hippuric acid did follow the giving of alanine and benzoic acid whereas no such increase was found when ammonium carbonate was given under similar conditions. There is very definite evidence, however (McCollum and

Hoagland [1913] and Lewis [1914, 1, 2]), that the hippuric acid nitrogen is derived from nitrogen which is normally eliminated as urea. It has been suggested too by Friedman and Tachau [1911] and Lachner, Levinson, and Morse [1918] that the liver is in some way implicated in the formation of the glycine. Cohn has suggested that the glycine arises in part by the union of acetic acid and ammonia. Magnus-Levy, however, does not consider such a synthesis very probable. Sassa [1914] also could obtain no evidence of the synthesis of glycine from glyoxylic acid and ammonia, and Haas [1916] had negative results with amino malonic acid which readily yields glycine *in vitro*. He also showed that glutamic and aspartic acids were without influence on glycine formation.

Can a synthesis, however, of a more complex amino acid take place in the same fashion? As has already been shown (p. 71) the only evidence in favour of such a synthesis rests on a false analysis. Against such a synthesis are the long series of experiments on the value of lysine by Osborne and Mendel and the feeding experiments with gelatin, which lacks most of the aromatic group of amino acids, and with zein, which lacks glycine, lysine and tryptophan. It is impossible to use either of these substances as the sole source of nitrogen for the body, and therefore we must conclude that the tissues cannot have an efficient mechanism for the conversion of lower amino acids into higher. It must not be forgotten that Ackroyd and Hopkins [1916] brought forward a certain amount of experimental evidence that in the case of arginine and histidine, if there is not a conversion of one to the other, at least these two diamino acids can replace one another with a considerable degree of efficiency in a diet.

The great weight laid upon the experiments which are believed to indicate that a synthesis of the higher amino acids is decidedly improbable is not quite fair. It is true that the aromatic groups apparently play a special rôle in the tissues, or the mechanism which controls their breakdown (perhaps also their formation) is a specialized one, as shown by the anomaly in metabolism of alkaptonuria, but, on the other hand, it is possible that those proteins, which lack certain groups, although they have a dynamic value are not able to form ordinary tissue protein, but are able to form a protein or a protein-like body of a simpler type like protamine or histone? Such a substance might be retained in the tissues either as a protein reserve or as store material for building up and repairing new tissue when the necessary amino acids from some other source, e.g. a fresh intake of food, are available.

Formerly it was believed that Miescher's [1897] experiments on the Rhine salmon during their stay in fresh water at spawning time afforded absolute proof of the change of simpler monamino acids into the more complex diamino acid, arginine, which was required in large amount for the formation of the protamine found in the genitalia of the salmon. Miescher found that during the growth of the testes and ovary there was a great wasting of the muscle tissue, but did not believe that it contained sufficient preformed arginine to supply the needs of the new forming protamine. Weiss, however [1907], in Kossel's laboratory, demonstrated quite clearly that the salmon muscle contained an amount of arginine which amply sufficed for the formation of the protamine. In other words, a mere transference of arginine took place in the body without the necessity of any synthetic action. Dunlop [1898] has also shown that all the protein nitrogen lost by the salmon during the ascent of the rivers for spawning is not required for the formation of the testes and ovary. It may therefore be assumed that the whole change which takes place in the salmon during the development of the genitalia is a mere change of position rather than the result of synthetic action. Noël Paton [1898] has shown that the amount of fat which the salmon accumulated in its muscles during its sojourn in the salt water is not only amply sufficient to yield all the fat required by the growing sexual glands, but is also sufficient to yield the energy for an enormous amount of muscular work. In this way the protein of the muscle tissue, apart from the ordinary wear and tear, is not called on to supply the energy required for movement. It must also be remembered that the salmon starts from the estuary for its ascent of the river with a very large store of some soluble protein in its tissues, and that this steadily diminishes in amount (Boyd [1898]). Is this soluble protein a highly specialized one eminently suited for the formation of protamine or is it ordinary protein filling out the cells and which is used either for energy or building purposes? C. W. and C. H. Greene [1919], who have carried out a very thorough investigation into the changes in the constitution of salmon muscle during the journey from the sea to the spawning beds are firmly of the opinion that there is a real storage not only of fat but of protein in the muscle fibre. C. W. Greene's estimate of the lecithin content leads him to the conclusion that ovarian lecithin comes directly from muscle lecithin. He is also strongly of the opinion that during migration the main source of kinetic energy is the intermuscular fat which falls to a low level but that the interfibrillar fat must have some other function as it remains practically constant throughout the migratory period.

On the other hand, the evidence adduced by Henriques and Hansen [1904, 1906] in connexion with an entirely different question must not be forgotten. These workers stated that they were able to get rats into a state of nitrogenous equilibrium when they fed them solely on the monamino acid fraction of a digest. Now if these experiments be accepted then we must at the same time accept the synthesis in the organism of the diamino acids, not to speak of other complex nitrogenous bodies precipitated by the phosphotungstic acid, from simple amino acids and simple peptides. These experiments are not beyond criticism as the evidence that the diamino acids were completely removed is far from conclusive.

Again, in the experiments which have been carried out where asparagine has formed the sole source of the nitrogen supply (see Chap. III., p. 44), synthesis not only of the diamino, but also of the other monamino acids, aliphatic, aromatic and heterocyclic must have taken place. It may be that in these experiments the bacteria alter the asparagine before absorption from the intestine.

And finally the growth of moulds like *Aspergillus Niger* on a medium containing potassium nitrate or glycine as the sole source of nitrogen is evidence of the synthesis of amino acids as a protein of complex structure is formed from which glycine, alanine, leucine, glutamic and aspartic acids can be isolated.

CHAPTER VI.

PROTEIN REQUIREMENTS.

A QUESTION which must now be considered is that of the minimum amount of protein required daily by the body. The subject is one not merely of scientific but also of economic importance.

The Protein Minimum.

The term protein minimum may be used in two senses either with reference to the lowest output of nitrogen in the urine which can be attained by the ingestion of a nitrogen free diet (experimental minimum) or with reference to the smallest amount of protein which must be ingested not only to allow of a positive nitrogen balance but which will also maintain the organism in a sound physiological condition (practical minimum). The term in its latter sense will be mainly discussed in the present section and the bearing of the former will be found on p. 137.

One point must be made clear at the outset, namely, that the search for an absolute minimum is like the search of the philosopher for absolute truth. There is not one minimum but many protein minima—the minimum is a resultant of many factors. Rubner [1903, 1919], Caspari [1905], and others, also hold firmly to this opinion. Rubner has in a series of papers pointed out that the demand by the tissues for protein is not merely to serve one purpose, and that therefore it is logical to refer to a variety of proteins, each of which may bring about alterations in retention and output. He has definitely laid down three purposes which have to be served by the protein ingested (1) to replace wear and tear, (2) for storage and growth, and (3) for dynamic purposes. When an organism is fed on a calorie abundant but nitrogen-free diet of carbohydrate the lowest limit in the output of nitrogen—the amount arising from the wear and tear of the tissues—is reached. If then it be desired to adopt a standard value for the term minimum protein this may be accepted. Osborne and Mendel [1916, 1] have emphasized the same point. Caspari quotes the work of Larguier des Bancels [1903] in support of the

multiplicity of the protein minima. The facts which can be cited against a common minimum are many in number. Thus the caloric value of the food given influences very definitely the protein minimum intake required by the organism. Also the nature of the foodstuffs fed with the protein influences the amount of nitrogenous material metabolized as is shown, for example, in the experiments of Voit and Korkunoff and many others. The temperature, too, as Rubner has shown, markedly influences the course of protein metabolism, and finally the activity of the organism plays a most material rôle.

Voit and Korkunoff [1895] were amongst the earliest workers who investigated this question. Taking the output of nitrogen of a dog on the third day of fasting as the standard for the amount of protein metabolized daily they found that the amount of nitrogen required to prevent loss of nitrogen from the body varied very markedly with the nature of the diet, i.e. whether it consisted of pure protein (washed flesh), or protein plus carbohydrate, or protein plus fat. The physiological protein minimum was always found to be greater than the amount of protein catabolized in the tissues during hunger. Thus for every 100 gms. protein catabolized in starvation 368 gms. of pure protein, 157-193 gms. of protein when mixed with fat, and 108-134 gms. of protein when mixed with carbohydrate, had to be consumed in order to prevent loss of nitrogen. Thus the addition of fat lowered the amount of protein required from 368 gms. to 157 gms., a decrease of 57 per cent., and the addition of carbohydrate reduced it from 368 gms. to 108 gms., a decrease of 70·6 per cent. Cremer and Henderson [1901] repeated some of this work, but they were unable to obtain the extreme values of Voit and Korkunoff. Michaud [1909], who also dealt with this problem, attributed the previous failure of workers to get nitrogenous equilibrium with the fasting nitrogen output to the fact that the protein fed differed too markedly in composition from that of the tissue protein. He stated he nearly reached the hunger minimum if he fed a protein which was of exactly the same constitution as the tissue protein, i.e. if he fed dogs on dog's flesh. As a result of his experiments, he drew up a scale of the amounts of protein required. This scale varied according to the similarity in composition of the protein to that of the tissue. Flesh of another animal—horse flesh—fed to a dog was less valuable than dog flesh, caseinogen was still less, and vegetable protein the least. He did not believe that the apparent unsuitability of vegetable protein depended on the lack of extractives present in these proteins. Zisterer

[1910] published experiments which substantiate this hypothesis of Michaud. He agreed with the general results reached, although he thought that Michaud was not careful enough in establishing his hunger value. Zisterer's figures for the ratios of the different protein material as regards the minimal requirements were as follows:—

Dog's Flesh.	Horse Flesh.	Nutrose.	Caseinogen.	Edestin.	Gliadin.
100	108	121	128	153	163

These figures bear out the conclusion reached by Michaud, that an animal can subsist on an intake of protein which is lowest when the protein given resembles most closely the tissue protein. Frank and Schittenhelm [1910] have carried out some work along the same line as Michaud. They found that all their dogs did not give the same results. Thus one dog did not reach the lowest level of nitrogen intake with the dog preparation, whereas two other dogs most unmistakably showed a diminution in nitrogen output when placed on dog flesh. They also tested a boy with goose, fish and ox flesh, and found that goose flesh took first place, and fish flesh next. In a later series of experiments [1911] they came finally to the conclusion that Michaud's deductions were not correct. They found practically no difference in the retention of nitrogen (after a prolonged low protein diet) after dog or horse or ox flesh. Dry skim milk and fish were both efficient substitutes. On the whole abiuret digest products were not quite so efficient as the whole product. Hoesslin and Lesser [1911] criticized Michaud's work unfavourably and agree with the findings of Frank and Schittenhelm. Wolf [1914] has also dealt with this problem using dogs and feeding them on dog and ox flesh. His figures were to some extent in favour of the dog flesh—the nitrogen, sulphur and phosphorus balances were all greater during the dog flesh feeding period than the ox flesh period. Busquet [1908] in a somewhat similar set of experiments on frogs found that if frogs were fed with frog muscle the gain in weight by the animal was much greater than when they were fed with the same amount of veal or mutton. Thus he found an increase in weight in one month of only 2 gms. in the veal fed frogs, whereas those fed on frog flesh gained 12 gms. in the same period. Billard [1910] found that tadpoles fed on frog's liver developed better than those fed on calf liver or algæ.

Siven [1900, 1901] carried out a long investigation on the question of the possibility of reducing the protein intake employing an entirely different method. He experimented upon himself, using diets containing varying amounts of protein. He came to the conclusion that the

fully grown human organism, for a short period at least, and without any increase of the caloric intake over the normal, could remain in nitrogen equilibrium with an intake of nitrogen of 4·52 gms. (i.e. 28·3 gms. protein, of which only about 12·5 gms. was in the form of pure protein). If the amount be reckoned per kilo. of body weight then the nitrogen requirement is 0·08 gm. of which only 0·03 gm. requires to be in the form of pure protein nitrogen. This amount of nitrogen is considerably smaller than that which was excreted on the third day of complete starvation by Succi: it was not until the third week of fasting that values in any way comparable with the figure of Siven were obtained. It is certainly true that if at the close of a fast the subject be put on a protein-free diet the output of nitrogen—an output which may reasonably be taken to represent the real protein catabolism—may reach a still lower figure. Thus Cathcart found [1907] in the case of Beauté that the output of nitrogen in the urine on the third day after a fast of fourteen days when he was fed on a diet of starch and cream, amounted to only 2·84 gms. Landergren [1903], Folin [1905, 1, 2], and Cathcart [1909], have also obtained in the normal individual remarkably low outputs of nitrogen when the subject was confined to a diet rich in carbohydrate but practically free from protein. Far-reaching conclusions concerning the actual amount of protein required daily cannot be drawn from such experiments unless it be shown that these positive results are not due to the very short duration of the experiments. The importance which is to be attached to results obtained from feeding experiments of short duration, more particularly those in which the diet lacks one or more of the normal constituents or contains them in abnormal proportion or form, is still a subject of dispute.

The Work of Chittenden.

As regards the practical protein minimum for an everyday dietary there is a great difference of opinion, more particularly since the interesting work of Chittenden was published. Voit had laid it down as the result of repeated experiment and observation that the daily intake of protein should be about 120 gms.

Chittenden [1905] held that this amount was both extravagant and dangerous. He was able to maintain nitrogen equilibrium on diets which contained about 6 gms. of nitrogen, equivalent to some 40 gms. of protein, and which were in addition of very low caloric value, 27 to 28 calories per kilogram. Chittenden's investigations were not merely confined to experiments on himself but were arranged on a large scale

and carried out on three different classes of men: (1) professional men engaged for the most part in laboratory work, (2) on student athletes who were in training for various university contests, and (3) on soldiers who performed a series of regulated exercises daily. The period during which these men were under investigation was also a prolonged one, thus giving the experiment a good chance of failure. Chittenden maintained that the results were excellent both physically and mentally in all classes of individuals on an intake of protein on the average about half that of the so-called Voit standard.[1]

As a matter of fact the intake of protein in the diet of the average individual, as evidenced at least by the output of nitrogen in the urine, is not so large as is commonly believed. It has become a habit to assert that the average intake of protein in the food of the average man is large, but this assertion is probably not true, particularly in the case of those who follow sedentary occupations. Thus Hamill and Schryver [1906], for example, examined the urine of seven individuals in the Physiological Laboratory of University College, London, who throughout the period of examination carried on their usual duties, no care being exercised in the choice of food, and they found that the output of nitrogen corresponded to an intake of some 90 gms. of protein per diem. From my own experience this amount is probably a good average value.

In one recommendation at least Chittenden is absolutely at fault. He recommends a dietary containing 50 gms. of protein and of about 2500 calories as sufficient for a soldier doing hard work. This is quite an inadequate diet when judged by the standard laid down by Melville [1910] as the result of observations on soldiers under service conditions. Melville found that his soldiers did well on a diet containing 190 gms. of protein and of about 3400 calories. "I have no doubt in my own mind," he writes, "that this allowance is ample, and if it errs does so on the side of generosity." But on the other hand he thought that 145 gms. of protein in a diet containing 3500 calories was as low as it was advisable to go, "and might well be increased especially when hard work is demanded of men under conditions of exposure". In the late war the caloric value of the field ration, of all the allied armies at least, exceeded, in some instances, the United States Army for example, markedly exceeded this figure of Melville, and the demand for protein was very high. See also Cathcart and Orr [1919]. But even apart from these practical observations in the field it can be shown by

[1] An excellent summary of this work will be found in Mendel's essay in the "Ergeb. d. Physiologie," 1911.

direct estimation and calculation of the amount of work done by Chittenden's soldiers that the 2500 calories were quite inadequate for the work done. The simple fact that some of the soldiers lost as much as 8·5 kilograms in weight during the period of the experiment, which lasted about six months, showed that as the material for the supply of energy was not obtainable from the food it must have been drawn from the subjects' tissues.

Further, the work of McCay [1908] on a people—the Bengalis—who naturally conform to the so-called Chittenden standard—shows that as compared with races which have a higher intake of nitrogen they are of inferior physique, of low endurance and activity, and are not long-lived. Campbell [1919] carried out a most interesting series of observations on a number of medical students of different races at Singapore. The total nitrogen output was much smaller than that of the average European even in the case of those who consumed a mixed diet. His observations support the view of McCay that the races who live on high protein diets have a better physique and more energy.

Chittenden also showed that animals, dogs, fed for long periods on a diet containing a low proportion of protein did not die off suddenly, but on the contrary thrived. Benedict [1906] offered a trenchant criticism of Chittenden's work in which many facts against the propriety of a low protein intake were pointed out, e.g. those of Haecker, who showed that the resistance diminishes if an animal were kept for a prolonged period on a low protein intake. Two herds of cows, ten in each, were under observation for three years, one herd being fed on a diet containing the normal proportion of protein, and the other on a diet poor in protein. No marked difference was observed between the two herds at the end of the second year, except that those on the low protein diet were somewhat less in weight; during the third year the animals fed on the low protein diet began to fail, and as they became so ill the experiment was discontinued. Reid Hunt [1910] also showed that restriction of diet played a most important part in the variation in resistance offered by animals to certain toxic substances. Munk [1893] was also of the opinion that a restricted protein intake definitely diminished the powers of resistance of animals. Aron [1911] in the course of an interesting paper on the relation of the necessary intake for growth and maintenance cites a paper of Waters in which he showed that if a restricted diet were given to calves although limited growth took place the flesh remained "veal" whilst that of control animals of the same age became "beef".

Jägeroos [1902] has also investigated the problem whether the

organism suffered either directly or indirectly by living on a minimal protein intake; two pregnant bitches, in both of which the lowest limit of nitrogen exchange was about 0·2 gm. per kilo., were studied; one lived for about ten months and then died suddenly, probably from some infection following abortion; the other also died suddenly after about six and a half months. (Both animals were on the diet before pregnancy commenced.) In each the food was well utilized throughout, and the general condition continued excellent almost up to the end. He discussed the question whether death was due to lack of resistance towards infection induced by the nature of the diet, or whether the resistance was lowered by the unnatural life (in the cage) and the lack of exercise. (Confinement in cages is frequently accredited with producing failure in nitrogen retention.) He concluded that the fatal results were not due to low protein intake, that the dangers commonly associated with such a scheme of feeding were greatly over-rated, and that if a diet fulfilled all "hygienic" conditions as to amount, digestibility, etc., little attention need be paid to the amount of protein present—as long as there is enough to satisfy the tissue needs.

Quantity or Quality of Protein.

It will be shown later (p. 90) that a rapid elimination of nitrogen follows immediately on the ingestion of protein. This being so, why should so high an intake of protein be regarded by Voit and others as essential? What is the real daily protein requirement of the body? It is evident that the tissues require a fairly abundant supply of protein for purposes of repair, but it is not yet clear what exactly the daily needs are. As has already been pointed out (p. 63), the tissues evidently exert a certain selective action as in the gliadin feeding experiment. Again the different tissues differ in composition, and presumably require a varying supply of protein in order to satisfy the different repair requirements. As it has not yet been definitely proved that one amino acid can be changed into another if we give a low intake of protein we may be either starving the animal in whole or in part. To put it in another way, if we remember that the protein molecule is built up of a series of amino acids which for the present we may call A, B, C, D and so on, and if on a certain day the body requires for purposes of repair of a certain protein tissue x amount of amino acid K, it does not matter apparently whether the body in getting this amount of K has to discard ten times x amount of say amino acid B, and thirty times x amount of amino acid M. As Zisterer [1910] has pointed out

(p. 76), supposing syntonin is the substance to be formed and caseinogen be fed, then in order to obtain the necessary amount of alanine required in this synthesis between four and five times more caseinogen is required than if syntonin itself be given or, in other words to yield the necessary amount of preformed alanine for 100 gms. syntonin, 444 gms. caseinogen must be consumed. Zisterer, of course, in his calculation assumes that the amino acids required are present in the proteins and are not synthetized.

In this connexion the interesting work of Thomas [1909] on the "biological" values of a large variety of proteins is of considerable importance. This worker reduced his nitrogen output to a low level by the consumption of a protein-poor, followed by a protein-free, carbohydrate diet, then he superimposed the various protein materials for varying periods. He accepted Rubner's generalization that when the minimum nitrogen output was reached by a course of feeding with nitrogen-free food the nitrogen excreted arose solely from wear and tear, none of the protein having been used for dynamic purposes. His aim was then to find out how much exogenous protein was required to cover this deficit. His term biological value indicates the amount of tissue protein nitrogen which can be formed from 100 parts of food protein nitrogen. In making his calculations he used three separate variants of one formula. (He took the output of nitrogen on the last day of the nitrogen-free food as his figure for "urine N" in the formula):—

$$\text{A.}\quad 100\,.\,\frac{\text{Urine N (N free food)} + \text{balance}}{\text{N absorbed}}$$

$$\text{B.}\quad 100\,.\,\frac{\text{Urine N (N free food)} + \text{fæces N} + \text{balance}}{\text{N intake}}$$

$$\text{C.}\quad 100\,.\,\frac{\text{Urine N (N free food)} + \text{balance} + 1{\cdot}0}{\text{N intake} - \text{fæces N} + 1{\cdot}0}.$$

The first formula was used for general calculations but, although the results on the whole are quite satisfactory, as it ignored the fæces, the second was the one selected for the most accurate assessment of the biological value. The third calculation was only used when proteins were fed which gave rise to much waste and when the fæcal nitrogen exceeded 1 gm., as in the case of certain vegetables.

As the results of his investigations he obtained the following percentage values, i.e. the figures given mean that 100 gms. of the particular food protein nitrogen can spare the amounts of tissue protein nitrogen as detailed. The percentage of the nitrogen in the different food-stuffs which exists in the form of protein is given for purposes

of comparison. The values which Thomas gives as the true biological values are given in black type :—

	Calculation.			Percentage of T.N. in form of Protein.
	A.	B.	C.	
Ox flesh	105·73	**104·74**		87·50
Milk	99·65	**99·71**		95·03
Fish	92·33	**94·46**		93·72
Rice	84·63	89·63	**88·32**	95·00
Cauliflower	79·15	—	**85·83**	—
Crab	72·99	**79·15**		69·12
Potato	71·65	83·18 **[78·89]**	74·97	63·00
Casein (digested)	66·69	**70·14**	67·12	—
Cherries	66·42	—	**78·57**	—
Spinach	64·50	—	**63·83**	76·90
Nutrose	63·43	**69·02**	—	—
Yeast	55·02	71·91	**70·52**	—
Pease Meal	50·61	59·89	**55·51**	90·00
Flour	33·29	42·44 **[39·56]**	42·95	71·40
Maize	18·40	**[29·52]**	34·99	94·80

It is very interesting to note that although ox flesh has only some 88 per cent. of its nitrogen in the form of protein yet its biological value is nearly 105. Thomas lays stress on this point, and insists that the extractives seem to play an important part in the utilization of protein. But it would also appear that the nature of the extractives present must play an equally important part because crab tissue which is rich in extractives, largely it is true of a comparatively simple type, is less effective than fish tissue, which contains very little extractive, or milk which contains even less. It is also of considerable interest that the effect of previous digestion exercises but little influence on the biological value.

Boruttau [1915], in view of the very low values obtained for vegetable foods, carried out a further series of experiments using the methods of Thomas. He also found low values but, arguing that it was highly improbable that the herbivora should feed in such a wasteful fashion, he put forward the view that in vegetables, prepared for human use, material, which normally augments the biological value, is discarded ; material which, under natural conditions of feeding, would be consumed by the herbivora. Osborne and Mendel [1915, 2] in their series of experiments on growth and maintenance found very marked differences in the protein minima, thus much smaller amounts of lactalbumin than of casein, edestin or gliadin sufficed to keep rats in maintenance. They also [1916, 2] noted in a series of experiments, in which the various proteins were fed in precisely equivalent and

increasing amounts in accord with the anticipated needs of the increasing body weight, that lactalbumin gave the best results. The effect of lactalbumin and casein were closely studied and it was found that, if to the casein diet there were added the appropriate amount of cystine, the results were as good as those with lactalbumin. They definitely state that this is a specific result due to the addition of the cystine and is not merely one due to the stimulating effect of amino acids in general, as the addition of the same amount of alanine under precisely similar conditions failed to induce more rapid growth.

As already referred to the intramolecular arrangement of the protein fed seems to play a part. Very similar to the work of Dakin and Dudley is that of Müller and Murschhauser [1919] who found that although ordinary casein was utilized to the extent of over 96 per cent. the same casein hydrolyzed by means of hydrochloric acid was utilized to the extent of nearly 99 per cent., but if the hydrolysis were carried out by means of sodium hydrate the utilization fell to below 60 per cent., and if more completely hydrolyzed by heating with sodium hydrate under pressure of four atmospheres the utilization fell to below 40 per cent.

Obviously, it is not so much the quantity but the quality of the protein given which is of prime importance, and it follows that on the whole it is safer to give a relatively large intake of protein than a small one. There is the further consideration that in order to obtain the requisite daily caloric intake protein must be taken in fair amount. There is a definite limit to the intake of both carbohydrate and fat on account of the difficulties of digestion attendant on too full meals of these substances. At most carbohydrate and fat supply only about four-fifths of the caloric requirements, and the balance must be made up by the addition of protein.

Feeding Experiments with "Abnormal" Proteins.

The study of the chemistry of the proteins makes it abundantly clear that proteins found in nature are not all of equal value. We have evidence from the analytical side and now a considerable amount of evidence of the most interesting kind is accumulating to show that even proteins which appear to be chemically identical may differ markedly when tested biologically, thus the racemization experiments of Dakin and Dudley [1913, 2], Dakin and Dale [1919] and of Dudley and Woodman [1915], and those of McCollum and Davis [1915] and of Geiling [1917], on the effects of heat on the nutritive value of casein.

In the present section the experiments dealt with refer particularly

to the imperfect proteins in the sense of there being essential amino acids either absent or present in insufficient amount. Although the classical example of gelatin has been known for many years the real bearing of the experiments do not seem to have been appreciated until Hopkins, as the result of his experiments with zein, suggested that certain materials taken in the food are essential to the organism and can be utilized without in the slightest degree contributing to tissue formation or structural maintenance; the formation of adrenaline from some of the aromatic nuclei of the protein molecule is an instance. That such a formation may take place has been suggested by Halle [1906] who stated that after digesting suprarenal pulp with tyrosine more adrenaline could be obtained than from control experiments carried out without such addition. This work was questioned by Ewins and Laidlaw [1910] who found no evidence of the conversion of tyrosine, or even of the more closely related bases, parahydroxyphenylethylamine and dihydroxyphenylethylmethylamine, into adrenaline by ferment activity.

It is, of course, a well-known fact that the members of the aromatic group of amino acids and cystine are practically completely absent from the gelatin molecule. Feeding experiments have been carried out with gelatin, and with gelatin plus the missing amino acids, but complete replacement of protein has not yet been achieved. Whether this be due simply to the amino acids not being added in proper amount, or in proper combination, or whether it be due to the absence of some other essential substance is not clear. Kaufmann [1901] carried out very complete experiments on himself and dogs. He found, like many others, that only a comparatively small amount of the protein can be replaced by pure gelatin (in dogs between one-fifth and one-fourth of the protein of the diet). He stated, however, that if he replaced the caseinogen nitrogen in his own diet by 93 per cent. of gelatin nitrogen plus 4 per cent. tyrosine nitrogen, 2 per cent. cystine nitrogen, and 1 per cent. tryptophan nitrogen, the mixture almost sufficed to prevent protein waste (no trace of the amino acids was found in the urine). In the case of two dogs which he also tested, he found that from one-half to one-third gelatin nitrogen plus 4 per cent. tyrosine and 2·5 per cent. tryptophan partially prevented loss of tissue protein. Murlin [1907] held that, quite apart from the addition of these missing amino acids, the retention of nitrogen during gelatin feeding depended to a large extent on the nature of the remainder of the diet. He believed that the presence of carbohydrate in large amount was of paramount importance. If carbohydrate were given

with the pure gelatin it was possible to replace, for a short period at least, 63 per cent. of the total nitrogen, and even to obtain a small retention of nitrogen. Rona and Müller [1906] came, on the other hand, to the conclusion that the capacity of gelatin to replace protein was not enhanced by the addition of 4 per cent. tyrosine, and 2·5 per cent. tryptophan. In only one of their experiments was there the slightest evidence of increased sparing of protein due to this addition. About two-fifths of the protein nitrogen could be replaced by gelatin with the addition of the amino acids. Totani [1916] found in his experiments with gelatin that minimal traces of tyrosine seemed to be effective. He thought it probable that the tyrosine was supplemented by phenylalanine. Abderhalden and Bloch [1907] found that, in the case of alkaptonuria, if the amino acids (tryptophan, cystine, tyrosine, phenylalanine, leucine, alanine, glutamic and aspartic acids) were added to the gelatin to make up the deficiencies, about one-half of the protein could be replaced.

The experiments of Hopkins and Willcock [1907] on the protein-replacing power of zein, a protein which contains no tryptophan in its molecule, yielded evidence similar to that obtained from the gelatin-feeding experiments. These observers fed mice on a diet of zein, carbohydrate and fat. They found that 77 per cent. of the mice died within twenty days. After the addition of tyrosine to the food 70 per cent. died within the same period, and after the addition of the missing tryptophan only 20 per cent. died in the twenty days, nearly 70 per cent. of the remaining animals lived over thirty days. Not only did the mice fed with the zein and tryptophan live longer, but their physical condition was much better than that of the other two series of animals. As the result of these experiments Hopkins [1906] was led to postulate the presence of certain essential substances in the diet to which he gave the name of accessory substances. The value of tryptophan has been repeatedly shown and the experimental results amplified by many workers (see p. 41). It is interesting to note that Asayama [1916] found that kynurenic acid, a quinoline derivative of tryptophan, could not replace that amino acid in the diet.

Starting from these experiments of Hopkins, Osborne and Mendel have greatly extended the problem of accessory substances. Thus [1914, 1] they found that when an animal was fed on protein, poor in lysine or lysine free, growth came to a standstill, and when lysine was added to the diet either by the addition of the amino acid itself or as a lysine rich protein, like lactalbumin, growth continued. As they remark "it is a teleologically interesting fact . . . that those proteins

like casein, lactalbumin and egg vitellin which are in nature concerned with the growth of animals all show a relatively high content of lysine." They also carried the Willcock and Hopkins experiments further by showing that although the addition of tryptophan alone to the zein sufficed to keep the mice alive, if lysine were also given they not only remained alive but grew.

$$\underbrace{\underbrace{\text{Zein} \;+\; \begin{array}{c}\text{Tryptophan}\\ \text{3 per cent. of}\\ \text{the zein}\end{array}}_{\text{Maintenance}} \;+\; \begin{array}{c}\text{Lysine}\\ \text{3 per cent. of}\\ \text{the zein}\end{array}}_{\text{Growth.}}$$

In connexion with these growth experiments they made a most interesting observation [1914, 2, 1915, 3], namely, that although actual growth may cease due to a deficient dietary yet the capacity to grow remains latent and apparently unimpaired even after 532 days, i.e. after more than half the average duration of life of a rat (a large proportion of laboratory kept rats die within 600 days). They also noted [1915, 1, 1916, 2] that rats apparently limit their feeding to the amount of food yielding approximately the requisite energy. They found [1916, 1] that in order to obtain satisfactory growth lysine to the extent of some 2 per cent. of the protein must be present in the food. With zein as sole protein they found that tryptophan equal to 3 per cent. of the zein consumed must also be added.

In another series of experiments Ackroyd and Hopkins [1916] showed the absence of arginine and histidine from the diet bring about a very rapid loss of weight, but that when one or the other of these amino acids is present conditions remain approximately normal. They concluded, therefore, that these two amino acids can, with a considerable degree of efficiency, replace one another in a diet. In this conclusion they are supported by the experiments of Geiling [1917].

Lewis [1917] who experimented with dogs on a low protein diet found the addition of cystine to the food diminished slightly the nitrogen loss and therefore reduced the negative balance. He accordingly thought that cystine should be added to the list of amino acids for which there is a specific demand by the tissues. He found that neither glycine nor tyrosine added to the same diet under like conditions produced the result obtained with cystine.

The Influence of a "Pure" Diet.

It must be definitely understood that there is no intention of discussing here the significance of the recent and rapidly growing literature on accessory substances as a special monograph is to be devoted

to the subject. The present section is merely a brief summary of some of the facts which seem to me to have special interest.

Previous to the work of Hopkins [1906, 1907] a certain amount of work had been carried out on "pure" diets which is of considerable intrinsic as well as historic interest, for instance that of Lunin [1881], Hall [1896], Steinitz [1898], and Röhmann [1902]. At present the tendency seems to be to increase the number of accessory substances and to apply explanations, drawn from these deficiency investigations, in the most haphazard fashion to all sorts of conditions and to convert a valuable and interesting field of research into a happy hunting ground for the charlatan and the manufacturer of proprietary remedies. Occam's razor is still sharp and effective.

Recently McCollum and Pitz [1917] have very pertinently called attention to the magnification of these unknown substances pointing out that, even granting the presence of the well-established fat soluble A and water soluble B substances, unfavourable proportions among the well-recognized constituents of a diet, taken in conjunction with variable and unsatisfactory physical factors, as well as the part played by bacteria in the alimentary canal, are quite sufficient in themselves to "account for all observed types of pathological functioning which are referable to errors in diet". A careful study of the methods adopted by Osborne and Mendel for the elucidation of the problem would be most prophylactic.

Stepp has carried out a large amount of work which ostensibly is on the borderland between "pure" diet and accessory substances experiments. Thus [1909] he suggested that much of the earlier work on "pure" proteins was defective, and that the failure to maintain life was due to the lack of "lipoid" and not to the nature of the protein. He carried out a long series of experiments which clearly demonstrated that mice died when fed on milk bread, which had been extracted with alcohol, ether or sometimes with chloroform as well, whereas mice fed on the unextracted bread lived and thrived. The animals fed on the extracted bread ate with avidity for the first fortnight, but thereafter their appetites failed and they died. Another series of mice were fed on extracted bread plus the extracted material, but although the animals lived longer than mice fed on extracted bread alone they did not survive like the control animals. He held then that a certain amount of fat "lipoid"—which is not lecithin—was necessary in the diet. In this connexion the suggestion of Glikin [1908] is of interest that lecithin is a material of first-class importance in the tissue metabolism, especially in connexion with

growing tissues. Stepp carried out another series of experiments [1912] in which the effect of adding lipoid material to a lipoid-free diet was repeated. Alcohol-ether extracted food plus butter did not suffice, although the addition of raw milk to the extracted food did, but milk too failed if it were added after boiling. In [1913] he found that certain of his so-called vital lipoids were thermo-labile. He was strongly of the opinion that the animal body had no capacity for the synthesis of lipoid substances. McArthur and Luckett [1915] came to the conclusion from their experiments on mice that neither lecithin, cephalin, cerebrosides, cholesterol, nor fats are really essential, but that there is some unknown substance which must be present in any complete food. Later Stepp [1915, 1, 2] showed that there was no question of ascribing his results with lipoid-free feeding to the lack of accessory substances as the addition of material, accessory substance rich, did not prevent the early death of the animals. Finally [1915, 3] he showed that lipoid-free food could have its value improved by the addition of pure lipoids like cephalin, lecithin, etc., and almost fully restored by the further addition of accessory substances. Lipoid-free diets although not sufficient as diets can still be utilized by the body for dynamic purposes. Lipoid introduced parenterally (intraperitoneal or subcutaneous injection) could not replace lipoids in the diet although in beri-beri the parenteral injection of accessory substances was effective, and he accordingly deduced that the intestinal mode of absorption was absolutely essential.

Folin [1906] has suggested the existence of special tissue metabolisms which in all probability would necessitate a further supply of special food products for the maintenance of nitrogenous equilibrium in the living tissues. He suggests, for example, that creatine may be such a substance, and that in consequence muscle tissue is found to be rich in this material.

From the above evidence, it is clear that apart from the caloric intake and the protein, carbohydrate and fat content of the food, there is some factor or factors which influence the utilization perhaps also the amount of food required. The evidence now available undoubtedly points to the presence of some accessory substance or substances in normal food without which normal physiological activity is impossible.

The Psychic Influence.

Still another factor, of which at present we know but little, is probably of fundamental importance, namely, the psychic factor. Owing to the nature of the experimental diet it is frequently monotonous in

flavour, or may be even nauseous, and it causes a certain amount of reluctance on the part of the animal to eat it. This distaste leads directly to a failure in appetite which eventually results in faulty digestion and incomplete absorption and utilization. In other words, in these experimental diets the aim of the research may be vitiated simply by the lack of those stimuli which Pavloff has proved to be so essential for gastric digestion at least, and which in all probability will be found to be necessary for the other organs. McCollum [1909] investigated this question with a certain amount of success, and reached the general conclusion that the palatability of a diet was a very important factor. He found that, even with many of the simple mixtures of pure proteins, if sufficient care were taken to change the character and the palatability of the food, by altering the flavour for example, it was possible to induce an appreciable retention of nitrogen. Very young animals, probably due to the fact that their metabolism is so active and hunger so predominant a feature of their daily lives, are found to adapt themselves more readily than adult animals to a ration of a comparatively low degree of palatability. Knapp [1909] found, in a series of feeding experiments on rats, that although the animals at first greedily consumed their food, later, probably due to the monotony of the food, the animals ceased to eat and eventually died. Salkowski [1919] has pointed out that it is the common experience of all laboratories, where metabolic work is done, that many animals will rather starve than eat food they dislike.

The Rise in the Output of Nitrogen after a Meal.

It has already been mentioned, that soon after the ingestion of a protein meal there is a rapid rise in the output of nitrogen in the urine. This rapidity of output naturally leads one to doubt the necessity of a large intake of nitrogen. It has been repeatedly shown that after a protein meal there is an output of about 70 per cent. of the ingested protein within eight hours. This output does not occur in a single steady rise following the protein meal, but in a series of maxima, as a rule, three. Tschlenoff [1896], Veraguth [1897], and Hass [1908] all found that the first rise took place about the second hour, the second between the fifth and sixth hours, and the third, if present, about the seventh hour. In all probability the first rise is due to the increased blood flow causing a washing out from the tissues of accumulations of waste material; the second, and the third when present, represent the results of actual protein catabolism. The cause of the fall in output between the first and second rise may be due either to absorption and

subsequent deaminization proceeding slowly, or it may be that during this period the tissues are taking up the necessary nitrogenous material for purposes of repair, i.e. that a physiological retention occurs, and that the subsequent rise is due simply to the excretion of surplus and effete nitrogen. Pepper and Austin [1915] have shown that the non-protein nitrogen of the blood reaches its maximum about two hours after a meal, reaching normal again in from ten to fourteen hours. Graffenberger [1891] maintained that the rate of output varies very definitely with the nature of the nitrogenous material fed. Curiously enough a partially digested material like peptone did not cause so rapid a rise in the output of nitrogen as proteins like fibrin and gelatin, and although asparagine is a freely soluble substance it was retained for a considerable period in the body. Stauber [1910] on the contrary found that the maximum output when protein digest was given took place within the first or second hour. Van Slyke and White [1911] in their experiments with fish protein observed that the more rapidly and completely the protein is digested and absorbed the less is the retention. They believed that the maintenance of nitrogen equilibrium with digest products is less easy than with whole protein. In this they support the work of Loeb [1911] who maintained that a dog which could be kept in nitrogen equilibrium with 3 gms. of protein nitrogen had a negative balance when given 3 gms. of digest nitrogen. Koraen [1901] found in his experiments on feeding with protein (8·3 gms. nitrogen) that about 50 per cent. of the ingested nitrogen was excreted in five hours, and that up to 100 per cent. appeared within nine hours. When he lowered the nitrogen intake to 3·2 gms. all the nitrogen ingested had not appeared at the end of five hours. It is, however, extremely questionable if these percentage figures of Koraen are right. If, as seems probable from his figures, he has taken the endogenous nitrogen output along with his exogenous, on deducting the endogenous nitrogen figure (the fasting nitrogen output which he gives for the subject used) his nitrogen output during five hours amounts only to about 26 per cent. and for nine hours to a little over 50 per cent.

Mendel and Lewis [1913, 1, 2, 3] made a most valuable study of the diet factors which influence the rate of the elimination of nitrogen. They showed a delay took place if indifferent materials like vaseline, filter paper, cork, etc. (except sand), were added to the diet, due, in all probability, to delayed digestion from slow emptying of the stomach or to adsorption by the added indifferent material. But they also found that the addition of carbohydrates, both soluble and insoluble, led to a reduction in the rate of elimination; in this instance the

result is not due merely to digestive delay but also to the fact that carbohydrates are efficient sparers of protein and therefore the metabolic processes are probably involved. When fats were tested the results were not so satisfactory: there was certainly a slowing when fluid cotton seed oil was added, but with lard and oleostearin the nitrogen output in the early periods was more rapid than normal. And finally they tested the influence of the nature of the protein ingested and found that ordinary meat and extracted meat powder differ radically in their rate of elimination; the slower rate of excretion after extracted meat powder was probably due as much to the proportionately larger content in connective tissue as to the fact that it was a dry preparation and lacked extractives. They also found on testing other protein products that casein and ovovitellin behaved like extracted meat powder, whereas edestin, gliadin and gelatin were like fresh meat. Other proteins like raw egg white and ovalbumin were also tested but gave very variable results, due probably to differences in rate and completeness of digestion and absorption. They came to the general conclusion that leaving aside differences in digestion and absorption proteins do not differ materially in their rate of metabolism.

As regards the individual amino acids it has already been pointed out that Levene and Meyer [1909] and Miss Bostock [1911] among others have demonstrated that within a few hours (eight to nine) about 90 per cent. of the ingested nitrogen of alanine and glycine is excreted, for the most part, in the form of urea. Here again, as in the case of the protein, there is some variation in the rapidity of excretion depending on the amino acid used. For instance, in the case of leucine only about 54 per cent. of the nitrogen appears in the first twenty-four hours.

The Effect of the Partition of the Diet on the Output of the Nitrogen.

As Voit pointed out long ago the nitrogen output depends for the most part on the amount of nitrogen contained in the food consumed. The rate of this output seems to be influenced, however, by the partition of the food—the giving of the food in several amounts instead of at a single meal. Adrian [1893], [1894] found that this partition sometimes increased and sometimes decreased the amount of nitrogen excreted. The variation he observed was probably due to faulty methods. Munk [1894] also with poor methods found a slight increase in the nitrogen output, but Krummacher [1897], on the other hand, using a fairly

reliable method showed that the division into meals brought about a slight decrease due to the fact that there was a constant succession of maxima of absorption—a further supply of food coming in before the previous supply was completely disposed of, with the result that there was some slight decrease in the absolute amount of material metabolized. Gebhardt [1897] observed that the partition of the food into separate meals brought about some decrease in the amount of total nitrogen excreted, and Hoesslin and Lesser [1911] in their experiments on dogs also found that there was some reduction in the nitrogen output if the food were distributed over the day and not concentrated on one meal. In this connexion the work of Leathes [1907] is interesting. He showed that there was a well-marked tide in the output of total nitrogen; in the twenty-four hours the highest output was between ten p.m. and four a.m. and the lowest output between four a.m and ten a.m. A similar variation in output was also found with uric acid and creatinine. Osterberg and Wolf [1907] have confirmed this observation.

Storage of Protein.

Another question of great importance is whether protein is ever stored in the body in the same way or approximately the same way as carbohydrate and fat. No one doubts now that excess of carbohydrate from the food is stored in the liver and other tissues, principally the muscles, as glycogen or, if in great excess as fat, and that the fat is stored as fat. Both these stores can give up their supplies with readiness when required. But the question of protein storage has not yet been definitely settled. There is no doubt that the body requires daily a certain supply of protein material to repair tissue waste, but if there be no supply from without, can the body for a short period, as is the case with carbohydrates, or for a longer period, as in the case of the fats, draw this necessary protein from stored material, or must it actually break down its protein tissues. If the demand for endogenous protein be prolonged, as in the case of a fast or in protein starvation, then the protein for the most part comes from the breakdown of protein tissue. Voit and Chossat have both shown that there is a general wasting of certain protein tissues like muscle, during a fast. In this connexion the work of Boyd [1898] and Greene [1919] on the muscle protein of salmon is of interest. In spite of the long period of fasting and the diminution in nitrogen content there is apparently but little change in the structure of the muscle fibre (see also p. 101). But if the

deprivation of the supply of protein from without be only for a short period—a day or two at most—the answer is not so clear. Unfortunately the amount of experimental evidence in this field is not large. Pflüger [1903] as the result of observations, which he made during his well-known experiments on glycogen formation and storage, came to the conclusion that the liver must be regarded as a storehouse for protein. He held that the hepatic cells not only manufactured this reserve protein from the food proteins, but also that this protein, just as is the case with glycogen, was held in reserve in the liver to meet urgent demands from the active tissues. Schreuer [1905] by the study of the respiratory quotient, also came to the conclusion that there was a certain limited storage of nitrogenous material in the tissues. He suggested that after liberal feeding with protein food there was a temporary increase in the labile cell material in dogs. The only condition which altered the natural tendency to get rid of the stored material rapidly was if the increase in the protein intake was associated with an increase in muscle activity. Bornstein [1901] had previously reached much the same conclusion. He could always get a marked retention of nitrogen in the tissues if simultaneously with the increased protein supply muscular work was carried out. Thus when he added to a basal ration of 13·21 gms. nitrogen per diem 6·75 gms. nitrogen in the form of caseinogen, and at the same time did 17,000 mkg. of work daily, there was a definite retention of nitrogen, which he believed was in the form of flesh. The nitrogen retained was equal to about 800 gms. flesh; the calculated increase of weight during the period of experiment (eighteen days) amounted to 773 gms.; the actual increase, ascertained by weighing, was 800 gms. He concluded that if this "activity hypertrophy" were to take place readily then there must be an abundant supply of protein food. Atkinson [1918] was unable in short experiments to show that mechanical work had any influence on the hourly rate of metabolism of ingested protein.

Seitz [1906] has demonstrated, by actual analyses of the liver, that storage of protein material can take place in the hepatic cells. This worker carried out two series of experiments on hens and ducks. Four birds in each experiment were starved for eight days, then two were killed and the livers immediately analysed, whilst the other two were fed on cod flesh free from both fat and glycogen.

Exp.			Liver. Weight in Gms.	Liver. N. in Gms.	Liver. N. in Per Cent.	Liver N. in Per Cent. of the Total N. Content of the Bird.
I.	Starved hens	1, 2	34·241	1·1700	3·417	1·592
	Fed hen	3	54·060	1·4240	2·633	—
	„ „	4	50·822	1·5900	3·129	3·952
II.	Starved hen	1	26·024	0·8697	3·342	2·446
	„ „	2	24·262	0·8305	3·423	2·536
	Fed hen	4	57·298	1·7620	3·075	4·710
III.	Starved ducks	1	24·382	1·6690	3·353	1·853
		2	25·397			
	Fed duck	3	102·359	3·368	3·290	5·967
	„ „	4	89·092	2·838	3·186	5·151

These figures, although perhaps far from conclusive, show at least that in the case of the livers of the fed birds there is a greater content of nitrogen than in those of the starved ones. Of course this does not settle in any way the form in which the protein is stored. Schryver [1906], on the other hand, who examined the livers of fasting cats and those killed about five hours after a full meal, obtained results exactly contrary to those of Seitz. Schryver found that in practically every liver examined (fourteen on record, eight fasting and six fed) there was more total nitrogen present in the livers of the starving animals than in those of the fed ones. Not only was the total nitrogen increased under this condition, but there was also a slight increase of the non-coagulable, or residual nitrogen. It must be remembered that in Seitz's experiments the birds were carefully fed on a high protein diet for many days before they were killed, and further that retention could only be shown after such careful dieting. Schryver's experiments were not carried out to demonstrate this point, and therefore no care about the previous feeding was taken. Cathcart and Leathes [1905] have also shown that during active absorption from the intestine there is a definite amount of storing of nitrogenous material in the liver. Reach [1909] carried out a series of experiments to demonstrate this retention of protein by the hepatic cells, but the method he employed was quite different. He perfused the liver with a mixture of blood, Ringer's solution and a protein-iodine preparation. He found that usually there was a very marked retention of the iodine protein compound in the liver in the form of the iodine protein itself; very little breakdown of the protein took place as no free iodine could be detected. Kerr, Hurwitz and Whipple [1918, 1, 2, 3] have carried out a series of experiments in which they claim to have shown that the liver is intimately concerned in some way with the regeneration of the serum proteins

and consequently with their maintenance at a normal level. Injury of the liver by poisoning for example with phosphorus or chloroform is associated with or causes a slight fall in the content of protein in the blood serum. Grund [1910] who carried out some very careful experiments on the amounts of nitrogen and phosphorus in the liver and muscle of fed and starved dogs and hens, found that in the case of dogs the percentage of non-protein nitrogen to total nitrogen tended to fall both in the liver and muscle during starvation, whereas in the case of hens there was just as definite a rise. He found that the liver during feeding took up protein and in starvation gave it up more readily than muscle. He thought that there was at least the possibility of a certain amount of storage of protein but was of the opinion that such a store could not play any important part in the total metabolism. He found further, both in feeding and starvation, there was a general tendency, in spite of the limited alterations in the absolute amounts of N. and P., for the tissues to retain the same relative composition. Tichmeneff [1914] starved mice for a couple of days, and then, after feeding them for three days on ox flesh, killed them. A comparison of the liver composition of mice killed whilst fasting with those killed after feeding, showed that not only had the livers increased about 20 per cent. in weight, but that there was an increase of over 50 per cent in nitrogen content. He found a definite increase in the N. : P. ratio as the result of feeding.

A certain amount of histological evidence has been adduced in support of the view that the hepatic cells form one, at least, of the protein depots. Amongst the modern workers Boehm [1908], in an interesting investigation of the histological appearance of the hepatic cells after different kinds of food and digestion products, found that the size of these cells varied. The smallest cells were found after hunger, and the largest after protein feeding. He further found that the structure of the protoplasm differed with different foods. Feeding with alanine and asparagine had no definite effect on the hepatic cells. Reichenau [1909], maintained, however, that the histological evidence was valueless, that, for example, proteoses could not be shown to have any marked influence on the activity of the liver. Although Richardson [1915] has stated on the contrary that the addition of peptone or hydrolyzed casein to a hepatic perfusion fluid definitely inhibited the formation of glycogen. He also found, however, that the addition of erepton or an amino acid mixture (glycine, alanine, leucine, valine) was without effect. He further raised the question as to the form in which the protein was retained. Was it retained as protein or as protein

digestion products? Berg [1914] also by histological methods claimed to find that abundant droplets could be made to appear in the liver cells of salamanders and rabbits by feeding with protein. These droplets were neither found in the liver cells of starving animals, nor did they appear if the animal received an abundant diet of carbohydrate or fat. The droplets gave the Millon reaction. Berg and Cahn-Bronner [1914] repeated these experiments using a protein digest product (erepton) and obtained a similar result, and finally Cahn-Bronner [1914] using a series of peptones and proteoses obtained also positive results. The appearance, at least, of the droplets was the same no matter the nature of the protein or protein product fed. It is interesting to note that Cahn-Bronner was quite unable to obtain his histological evidence of storage when the protein was given intraperitoneally.

Johansson and Hellgren [1906] have also stated from experiments on human subjects that all food material, including protein, was not used directly it was absorbed, but that after absorption it was laid down in certain depots and only drawn on as required. The work of Gruber [1901] and Falta [1906] on the prolonged excretion of nitrogen which sometimes follows the administration of protein also supports the view that there is some storage of nitrogen. Abderhalden [1908, 4] produced a certain amount of evidence in its support. He found that a high intake of protein before a fast was not necessarily followed by a high output of nitrogen on the first day of the fast. He held, however, that the retention of nitrogen did not take place in a protein form but as "free" nitrogen, and he maintained that even in starvation there was an attempt by the animal to retain this "free" nitrogen. Morawitz [1906] has also given some contributory evidence in his work on the formation of the blood proteins. He found after thorough bleeding and replacement of the removed blood by a suspension of blood corpuscles in Locke's solution rendered viscid by gum, albumin and globulin did not reform at the same rate. The albumin gradually appeared in the first few hours after the bleeding, but the globulin much later. He concluded that the initial supply of albumin must have come from some store in the tissues. This reformation of blood proteins took place also during starvation, but in this condition he stated that the reformation of the globulin was if anything more rapid than the albumin.

Kerr, Hurwitz and Whipple [1918, 1] have definitely shown that the content of blood serum in protein undergoes but minimal fluctuations during periods of fasting or after heavy feeding. If, however,

the animal be frequently bled and the washed corpuscles, suspended in a gum Locke solution, be returned to the vessels (Abel's plasmapharesis) the normal content of protein can be reduced to a very low level. They found the regeneration of the protein after a 50 per cent. depletion is slow—seven to fourteen days. Further they observed that this regeneration could also take place in the fasting animal and therefore tissue protein must be capable of conversion into serum protein. During the regeneration there is practically no change from normal in the nitrogen output, therefore they conclude there is but little waste amino acids formed during the conversion. They noted that on the whole the globulin regeneration tended to be somewhat more rapid than that of albumin. They believe that their experiments definitely show that serum proteins are not intermediate products between food and tissue proteins. In another paper [1918, 2] they studied the effect of diet on the speed of regeneration and found that it was more rapid on a meat diet than on a bread and milk diet. Apparently the maximum regeneration during the first twenty-four hours is 1 per cent. of the total protein, a very constant figure which is but little influenced either by fasting or feeding. They make an interesting comparison between the rate of regeneration of tissue (hepatic) protein and serum protein. Whipple and Sperry found that after about a 50 per cent. reduction in hepatic protein due to chloroform necrosis it requires from seven to ten days for recovery, a figure very close to that for the regeneration of serum protein, and [1918, 3] they came to the general conclusion from these and other experiments that the liver is concerned in some way with the formation of serum protein.

Probably the only other conditions in which this retention of protein can be readily demonstrated (in addition to that caused by training or work already referred to) is during growth, pregnancy and convalescence from a wasting disease. Rubner [1908, 1912] has dealt exhaustively with the problems of retention during growth. He has shown how in the earliest period of post-natal growth the laying on of protein is associated with the diminution in the water content of muscle, basing his deduction on the interesting work of Thomas [1911]. In this connexion there are also the observations of C. W. Greene [1919] who found that with the diminution in the amount of protein in muscle tissue of salmon during the fast of spawning migration there was a rise in the water content. Rubner [1912] believes with Hertwig that although the actual protoplasm may play a definite part in the assimilation of food material yet the main site of the growth stimulus resides in the nucleus. He [1911] has also devoted

much attention to the reconstruction of the tissue after tissue loss—the protein used for filling out the depleted cell to its optimum condition he calls "melioration eiweiss". Above all he has emphasized the point that there is never so little protein available for storage as when the diet consists solely of protein.

There are also a large number of clinical papers bearing on the retention during convalescence, as for instance that of W. Hale White and Spriggs [1901] who showed that there was a daily retention of over 12 gms. of nitrogen, in a woman who increased from 39·2 kilos. (after inanition from worry) to 52·49 kilos. in fifty-five days.

Siven [1900] in his experiments has given a very practical example of the difficulty of bringing about protein storage in the body even when the conditions for such retention might have been held to be particularly favourable. After his feeding experiments on a low nitrogen diet, when he had lost almost 1 kilo. of muscle—32·41 gms. N., he suddenly increased the daily intake of nitrogen to 13 gms., but found after fourteen days feeding a storage of only 14·49 gms. N. and of this 11·93 gms. were retained during the first four days. He then raised the nitrogen intake to 22·5 gms. daily and found a retention of some 6·15 gms. nitrogen in six days, most of which was retained on the first day. In fact, on three days out of the six there was actually a negative balance. For the whole experiment there was thus only a retention of 20·64 gms. nitrogen, or about two-thirds of what the organism had previously lost, i.e. 32·41 gms. Apparently, then, tissue restitution must be a very slow process.

The only other strictly physiological condition in which retention of nitrogen would appear to take place with any readiness is that of pregnancy. Most of the earlier work must be discarded owing to the faulty methods utilized, but Hoffström [1910] has shown in a most careful piece of work that even in spite of a comparatively low intake of nitrogen there was a very steady daily retention of about 1·8 gm. (nearly 14 per cent. of the intake). In the course of the experiment there was a total retention of 310 gms. of which he believed 101 gms. were diverted to the fœtus. There was also a consistent daily retention of phosphorus. Hoffström held that pregnancy led to a definite storage of reserve nitrogenous material, only part of which was for the fœtus. Murlin [1911] also investigated this problem by modern methods and found (working with bitches) that the first half of pregnancy was characterized by a loss of nitrogen particularly during the third and fourth weeks but that during the second half a definite retention of nitrogen took place.

In what Form is Protein Retained?

An observation first made by Voit, and which has repeatedly been verified seems to be in direct contradiction to this apparent difficulty of protein storage. When the subject or animal under investigation is suddenly changed from a protein-poor to a protein-rich diet the nitrogen output does not immediately jump up to the new protein level; during the first few days there is less nitrogen excreted than is taken in the food, but eventually nitrogenous equilibrium is established. A similar result follows the change from a protein-rich to a protein-poor diet, i.e. during the first few days succeeding the change more nitrogen is excreted than is contained in the food. Two questions naturally arise in connexion with these observations. In what form is the protein or rather the nitrogen retained? and What is the cause of the retention? Rubner [1902] and Atkinson and Lusk [1919] have definitely shown that the moiety of the ingested protein which is absorbed and deposited as new protein, exercises no specific dynamic action and we must therefore conclude that this deposited protein goes out, so to speak, of solution and is for the time in a particularly stable state.

The first of these questions has been discussed by Gruber [1901], Falta [1906], Ehrström [1906], and others. The general opinion is that the retention takes place in the form of some protein-like material and not in the form of extractives or of end products. Van Slyke and Meyer [1913, 3] and Wishart [1915] noted for instance that although the ingestion of meat increases the amino acid content of the blood the muscle tissue shows no increase. Amino acids which are taken up by the tissues must either be synthetized to protein or destroyed. Greene [1919] has shown that when demands are made on the muscle tissue, as during starvation, the amino acid content may increase 100 per cent. from the breakdown of muscle protein. The amount of extractive or non-protein nitrogen in the body, however, is by no means an insignificant amount, as Schöndorff and others have shown. Thus Schöndorff [1897] found that the muscle tissue of a 22 kilo. dog killed after abundant meat feeding contained 40 gms. of nitrogen in the form of extractive bodies soluble in water and that there were from 15 to 20 gms. of urea present in the total organism. Van Slyke and Meyer [1913, 1] found as much as 80 mgm. amino acids per 100 gms. muscle.

Rubner [1904] and Burgi [1904] have stated that, in their opinion, part of the nitrogen from meat extract can be retained in the

body when the conditions are suitable as, for example, after the administration of the extract at the end of a period of starvation. They found that this retention was always associated with a great retention of water. As a rule, however, the nitrogen of the meat extract was rapidly excreted. Thompson [1910] has also shown that a retention of nitrogen given in the form of meat extract can occur. Voltz and Baudrexel [1911] showed that the addition of meat extract to a diet did not increase the absorption either of the nitrogen-containing or nitrogen-free moiety of the diet, although either a slight retention of nitrogen or perhaps prevention of nitrogen loss took place. They state that the physiological value of meat extract is only about two-thirds of its energy content. Thomas [1909], as already mentioned, has stated that in his opinion the extractives cannot be ignored as factors in nutrition.

The retention certainly does not take place mainly in the form of end or waste products such as urea, as it has been shown that if urea be given by the mouth it is quantitatively and rapidly excreted. This was demonstrated by Voit [1881] in the dog, and again by Achard and Paisseau [1904], who gave large doses of urea to a man and found that the output of nitrogen rose immediately and fell just as suddenly when the urea was stopped. Rosemann [1898], however, was inclined to believe, from his experiments, that a retention of nitrogen in the form of end products could take place. His investigation was carried out on a subject who could scarcely be regarded as normal and his results remain unsubstantiated.

As already stated in an earlier section, Abderhalden thinks it highly improbable that the retention occurs in the form of protein (p. 97). Von Noorden also holds that so long as the nitrogen retained is small in amount it is not necessarily in the form of tissue protein; one moiety of the retained nitrogen may be distributed in the blood and lymph and another moiety stored somewhere as reserve material. Pflüger held [1899] that if there were a retention of carbon there must also be a retention of nitrogen. He believed that a kind of intermediate substance was formed from the protein absorbed and stored as a reserve protein, a substance which was richer in carbon and poorer in nitrogen than the ordinary protein found in the tissues and tissue juices. This storing of material was an actual storage, i.e. it did not lead to a new formation of cells, but was a mere filling out of the cells already existent—a physiological hypertrophy—an eutrophy as it has been called. Even the activity hypertrophy has been stated not to be due to an actual increase in the number of tissue cells, but to a mere

thickening or distension of existent elements. Von Wendt [1911] from his own work came to the definite conclusion that the retention occurs as new material in the living cells and was not simply a mere retention of nitrogen in some intermediate form.

As the result of their study of the nitrogen phosphorus ratio in experiments where a great retention of nitrogen had taken place, Lüthje and Berger [1904] came to the conclusion that part of the nitrogen was certainly retained in the form of "flesh" and that the rest of it was probably stored in the form of dead intracellular protein just like so much glycogen or fat.

Pharmacology offers examples of a similar capacity for retention. Thus the retention of bromine, iodine and fluorine has been demonstrated by Tappeiner and Brandl (cit. Belli) and that of chlorine by Belli [1904]. In each of these cases the retention took place in the same step-like fashion characteristic of nitrogen. The selective action for iodine exhibited by the thyroid gland is even more striking.

What is the Cause of the Retention?

The cause of the retention is the need of the organism for nitrogen, but the laws which govern the demand and the nature of the retention and which regulate the rate and the manner of the output are not fully understood.

As an explanation of the nature of the retention Ehrström [1906] has suggested that there are certain "affinities" in the tissues to which the absorbed material attaches itself in a more or less labile union, this union lasting for very variable periods. The duration of the retention, he believes, is absolutely independent of the chemical form assumed by the retained material. On the other hand, Gruber [1901], without entering into any vague theorizing, sums up his opinion in the sentence "that this temporary retention of protein is simply the result of the superposition of the hourly curves". Assuming that the hourly curve of the output of nitrogen of one day is identical with the next (provided always that the protein given be similar in amount and nature), and that, as a result, the nitrogen from the daily protein catabolism is excreted in the same proportion in each 24, 48, 72 and 96 hours, Gruber has put forward an extremely interesting hypothesis which offers a very probable explanation, of the step-like increase or decrease in the output of nitrogen produced by variations in the protein intake (see Table). He suggested that 80 per cent. of the food nitrogen was catabolized on the day of ingestion, 13 per cent. on the day following, 5 per cent. on the third day, and 2 per cent. on the fourth.

Thus after an increase in the intake of protein there would be a steady step-like rise in the output of nitrogen until equilibrium of intake and output was reached. The same explanation holds good with a decreased nitrogen intake.

	Feeding Period.					Starvation Period.		
Days.	1	2	3	4	5	1	2	3
1	80	13	5	2	—	—	—	—
2	—	80	13	5	2	—	—	—
3	—	—	80	13	5	2	—	—
4	—	—	—	80	13	5	2	—
5	—	—	—	—	80	13	5	2
	80	93	98	100	100	20	7	2

Magnus-Levy [1907] has improved on this table of Gruber. He pointed out that even when the organism was in a state of nitrogenous equilibrium a certain amount of the protein ingested was required to replace broken down tissue protein. A certain amount was also retained in the organism to replace store or labile protein which had been utilized. In other words, the nitrogen excreted on a particular day did not wholly represent the nitrogen ingested on that day, part came from the food protein and part from the protein already present in the organism. Magnus-Levy, therefore, subtracted from the 80 parts of the nitrogen said to be disentegrated by Gruber on the day of ingestion the amount which would be required to replace the effete tissue protein. This amount he estimated as 30 parts.

This regularity of storage or excretion only occurs when there are no disturbing factors present. If, for instance, the subject had undergone a period of nitrogen starvation previous to his nitrogenous intake experiment then there would be very marked retention of nitrogen, presumably for purposes of repair.

The modification of the table by Magnus-Levy has been improved upon by Thomas [1909], as the result of his investigations into the biological value of proteins, so far as the nitrogen exchange is concerned on the giving of protein *after a protein-free diet*. Making the assumption that the food nitrogen is completely absorbed, he gives the following scheme of the probable conditions when the source of the nitrogen has a biological value (1) of 100 (2) of 40 :—

State of Nutrition.	N. Intake in Twenty-four Hour Period.	N. Excretion in Gms.		N. Balance in Gms.
		Urine.	Fæces.	
(*a*) Caloric sufficient but N.-free food .	—	3·0	0·5	− 3·5
(*b*) Ditto . .	+ 3·5 gms. N. in flesh (biological value 100)	3·0	0·5	± 0·0
(*c*) Ditto . .	+ 7 gms. N. in flesh			
(1) Previous N. loss	—	3·0	0·5	+ 3·5
(2) Without previous N. loss . .	—	3·0 to 6·5	0·5	+ 3·5 to 0·0
(*d*) Ditto . .	(1) + 3·6 gms. N. in flour (biological value 40)	5·2 1·6 from tissues 1·4 biologically available N from flour 2·2 waste from flour	0·5	− 2·1
	(2) + 6·0 gms. N. in flour	6·6 0·6 from tissues 2·4 biologically available N from flour 3·6 waste from flour	0·5	− 1·1
	(3) + 8·8 gms. N. in flour	8·3 3·0 biologically available N. from flour 4·5 waste from flour 0·8 waste for fæcal N.	0·5	± 0·0

All proteins are not broken down with equal readiness, and probably as a result there is considerable variation in the period in which nitrogen equilibrium is reached. Thus Falta [1906] superimposed different proteins on to a standard diet, whose effect on the metabolism was known, and found in his experiments a variation from about three to six days in the rate at which the nitrogen was excreted. This observation was made on man, but in the carnivora the differences in the rate of excretion did not hold good, or at any rate were much less marked. This step-like excretion is not due to slowness of absorption, as the excretion is spread over days, but must be due to a step-like breakdown of protein in the body. Falta has suggested that in the absorption of protein, part is taken up in some high molecular form, and part in a small molecular form. According to the immediate needs of the organism part is retained and part burnt, the low molecular form being much more labile than the high. Wolf and Osterberg [1912] as the results of their experiments gave general support to the conclusions of Falta. A certain amount of evidence in support of this step-like breakdown is available, and will be considered presently. In the light of Falta's results, then, it is not only the amount of protein present which influences the amount of nitrogen excreted, but the nature of

the protein given also plays quite as an important part. Tsuji [1915, 1] and Umeda [1916] have shown that the nature of the basal ration on which the protein is superimposed also plays a leading rôle. As Ehrström [1906] has remarked, the capacity which the body possesses of catabolizing protein is constant, not variable. The variable factor lies in the differing chemical constitution of the products of digestion and their varying resistance to such catabolic action.

Examination of the Nitrogen Sulphur Ratio.

Evidence of the rate of protein catabolism has also been put forward from quite another point of view, namely, from the investigation of the sulphur output. Sulphur, like nitrogen, is found in practically all proteins, but in more variable amount. It had been suggested that from the study of the ratio of the sulphur nitrogen output, evidence might be obtained of differences in the rate and form of the breakdown of proteins. This ratio has accordingly been fairly fully investigated by several workers. Von Wendt [1905] carried out some extremely interesting and careful work which demonstrated clearly that the amount of protein catabolized can be calculated quite as accurately from the output of sulphur as of nitrogen. Using as a factor 9·3—the ratio of nitrogen to sulphur excretion in a nitrogen starvation experiment—and multiplying the daily output of sulphur by it, figures were obtained which were practically identical with those obtained by the Kjeldahl nitrogen method. The drawback of course to the utilization of the sulphur output for estimating the protein breakdown is a technical one—the method of estimation is comparatively slow. Von Wendt held that it was only by the combined examination of the nitrogen and sulphur output that a true picture of the total protein exchange in the body could be obtained, individually they only indicated the excretion of certain decomposition products. He maintained that the tissues first burned and excreted the sulphur-rich digestion products of the protein, and that the nitrogen-containing material, which was retained in the body, was poor in sulphur. It was suggested that this retained material might be some decomposition product which, although it was not a true protein, could not be washed out of the tissues. Rubner in his experiments also noted that the sulphur output very frequently preceded the nitrogen output.

Gruber, on the other hand, maintained that the output of nitrogen and sulphur ran parallel, and concluded that the protein material stored for use in the tissues resembled normal protein. The experiments of Sherman and Hawk [1901] and of Siven [1900] support the view that

the output of nitrogen and sulphur run very nearly parallel. Ehrström [1906] also found that although generally the nitrogen and sulphur output run nearly parallel, the sulphur output on the whole adapted itself more rapidly to changes in the intake. He suggested that the protein products most deeply split were richest in sulphur, and possibly that they underwent oxidation in the tissues first thus accounting for the more rapid excretion of sulphur sometimes observed. His work, therefore, lends support to that of Von Wendt.

Hämäläinen and Helme [1907] investigated the rate of the nitrogen and sulphur output, using the superimposition method introduced by Falta in which the material to be tested is added to a standard diet. Their standard diet was one poor in nitrogen, and on this they superimposed at different times egg white, proton, and roast veal. Of the three diets apparently the veal alone was able to supply the deficiency in nitrogen and thus replace the wasted tissue protein. This result is in accordance with the later work of Michaud [1909] and Thomas [1909]. They considered their results supported the hypothesis of Von Wendt that the sulphur-rich products of protein digestion were more rapidly burnt and excreted than those which contained less sulphur.

Wolf and Osterberg [1912] using the superimposition method on man and dogs and with a wide variety of substances, ranging from ammonium citrate and urea to a series of proteins and amino acids, found that in many instances the sulphur seemed to belong to the labile molecule as it appeared before the nitrogen. They showed that this did not depend on the cystine group as it also occurred when gelatin was used. They found that the form in which the food was given materially affected the rate of excretion (see also p. 146). Cathcart and Green [1913], who also used the superimposition method on man, concluded from their study of the S. : N. ratio, that the output was due to the catabolism of the protein actually ingested in the experiment as the ratio on the day of ingestion approximated closely to that of the protein fed. Sulphur was on the whole more rapidly excreted than nitrogen; retention of part of the nitrogen was most marked when a low protein dietary was employed as the basal ration. This did not hold good when urea was superimposed, as about 95 per cent. of the ingested nitrogen reappeared on the day of administration, and the day following. They came to the general conclusion that the retained material was apparently stored in the tissues as a pabulum of uniform composition. Tsuji [1915, 1], who utilized the same methods on dogs, found that the conclusions of Falta were correct, viz. that the

rate of excretion of the catabolic products of protein depended on the nature of the protein fed. He, however, further showed that the rate at which these products were excreted depended in turn largely on the nature and composition of the basal ration, retention of nitrogen being most definite when a carbohydrate diet was used. Sulphur also (in two out of three experiments) was better retained on the carbohydrate diet. The greater part of the extra sulphur ingested was excreted as inorganic sulphate. He could obtain no evidence that the protein material retained by the organism was poor in sulphur.

Apparently, then, in addition to the splitting of the protein molecule into a nitrogen-rich and a nitrogen-free portion, there is a like separation of the sulphur-containing fraction. The early breakdown of the sulphur combination may be associated with the rapid increase in the output of carbon dioxide which has also been observed after injection of food materials (Frank and Trommsdorff [1902]).

Examination of the Nitrogen Phosphorus Ratio.

The rate of the output of phosphorus has also been frequently compared with that of the nitrogen output, and Grund [1910] has investigated the N. : P. ratio of tissues after varying conditions of feeding. It has been found that the phosphorus output does not bear the same intimate relation to the rate of protein catabolism that sulphur does. This is not a matter for surprise, as in all probability the phosphorus, or the greater part of it, is associated with the special nucleo-proteins of the food and the tissues, and may undergo a special form of catabolism. As will be shown later in discussing the course of catabolism during fasting nucleo-proteins apparently are more resistant to the action of proteolytic enzymes than the ordinary tissue protein. The curve of the phosphorus excretion has been found to run more or less parallel to the nitrogen curve, but behind it. The output of phosphorus has been investigated by Luthje and Berger [1904], Sherman and Hawk [1901], Siven [1900], Ehrström [1903], Tigerstedt [1904], Hämäläinen and Helme [1907], and others. It has also been shown that the animal organism does not require to receive its phosphorus in organic combination, that it can synthetize the various phospho-proteins and phospho-lipins from phosphorus supplied in an inorganic form (Holsti [1910], Rohmann [1914], Fingerling [1912], and Stepp [1915, 2]).

CHAPTER VII.

THEORIES OF PROTEIN METABOLISM.

MANY hypotheses have been advanced to account for the various changes observed during the course of protein metabolism, but until comparatively recently only two were really considered; those of Voit [1867] and Pflüger [1893]. Previously the theory advanced by Liebig was almost universally accepted. Liebig considered that the protein of the food was the one essential material, that it entered the organism without having undergone any very serious change during digestion, and that it immediately and directly replaced the effete material of the tissues. He certainly grasped the fact, however, that all food neither served the same purposes nor underwent the same transformations.

Voit.

Voit [1881] put forward in 1867 the view that the protein of the food after absorption circulated in the tissue fluids—became "circulating protein"—and was utilized (catabolized) by the living tissues without first becoming an integral part of them. This "circulating protein" was readily broken down, whereas the "tissue protein" was resistant. A certain amount of the tissue protein constantly died and was excreted, and was replaced by material drawn from the "circulating" or food protein. No chemical difference existed between the "circulating" and the "tissue" protein. One of the facts which led Voit to the conclusion that protein existed in two forms was that during starvation a bare 1 per cent. of the tissue protein of the body was broken down per diem, whereas if protein were fed in amount equal to 12 per cent. of the body protein the breakdown was fifteen times greater than in hunger. He was probably the first investigator who really grasped the fact that the cells were the important factors in controlling the rate and manner of metabolism. He also appreciated the part played in the metabolism of protein by the other, non-nitrogenous, food-stuffs; indeed he ascribed to their presence the capacity of the cells to take up and fix in the tissues the circulating protein.

Pflüger.

Pflüger [1893] subjected the view advanced by Voit to a very severe criticism. He thought that the food protein must first become an integral part of the living protoplasm before it could be utilized; in other words, the absorbed food protein, unlike the living tissue protein, was not readily catabolized. He believed that a fundamental difference in chemical constitution existed between the two forms of protein and that the greater lability of the living tissue protein was in all probability due to the presence of cyanogen radicles in it. Verworn [1899] was inclined to agree with these views, but Vernon [1907] could obtain no experimental evidence which supported them.

Pflüger based his views largely on some experimental work carried out in his laboratory by Schöndorff. Blood was taken from a starving dog and was circulated through the hind limbs and liver of a *well-fed* dog; the urea content of this blood was increased at the end of the experiment. Blood was taken either from starved or well-fed dogs and circulated in the same fashion through the hind limbs and liver of a *starving* dog; no increase in the urea content was detected. Folin [1905] severely and justly criticized these experiments, pointing out that the evidence furnished by them was by no means unassailable. He worked out the details of one of Schöndorff's experiments, of which full protocols were given and in which an increase of urea in the blood to the extent of 125 per cent. was stated to have been found. Folin showed that the actual amount of catabolism during the four and a half hours of the experiment corresponded only to 25 mgm. of urea nitrogen. During the four days preceding the experiment the dog had catabolized about 35 gms. of nitrogen per day; this 25 mgm. gain therefore amounts to less than one-tenth of one per cent. As Folin says: "Considering the numerous sources of error and uncertainty necessarily attached to an experiment of this kind it seems very strange that the extraction of 25 mgm. of urea nitrogen from the hind legs of a dog killed while engaged in digesting 700 gms. of meat should be accepted as proving not only that protein catabolism did occur during the experiment, but also that it occurred in the bioplasm and not in the circulating protein".

Pflüger's theory does not satisfactorily explain the fact that very soon after the ingestion of protein there is a rapid rise in the output of nitrogen in the urine. One would have to assume an extraordinarily rapid synthetic process followed at once by an equally rapid catabolism. Pflüger looked on protein as the food preferred by the organism to all other foods and the one which it would assimilate most rapidly.

It seems improbable that all the nitrogen taken in is required or even utilized for protein synthesis. It is extremely difficult to obtain any direct evidence decisively in favour of one or the other of these very divergent theories. It is indeed highly probable that the differences which exist between the tissue and food protein are in degree and not in kind.

Rubner.

Rubner [1908] advanced a theory of protein metabolism, in which he maintained quite rightly that the study of metabolism could not be divorced from the study of heat production, therefore that metabolism must be considered in association with the energy exchange. He referred all the metabolic changes of protein to the production of energy. He believed in a "store" protein resembling Voit's "circulating" protein, which is simply a transitional form of food protein and in "a wear and tear quota" necessary for the repair of tissue waste. The greater part of the protein after absorption was rapidly disintegrated into a nitrogen-free and a nitrogen-containing part. The fate of the nitrogen-containing moiety, as it played but little part in the energy exchange, was disregarded. The nitrogen-free part of carbohydrate nature formed the dynamic quota of the protein ingested. In this splitting of the protein a certain liberation of energy—of heat—occurred which was of no value as a source of energy for the cellular activity, and was therefore lost. This liberation of energy was termed the specific dynamic action of the protein. He emphasized the point that in his opinion the nitrogen mass of the body controls neither the breakdown nor the synthesis of nitrogenous material. The total activities of the body as determined by the size and surface area of the animal are the sole regulators. Thus the smaller the animal the greater the breakdown of protein in starvation. Reconstruction follows the same law.

A highly speculative hypothesis explained how the various changes took place. All protoplasm was not regarded as being of the same type, one kind might be thermolabile, another thermostable, but all varieties had in common a certain molecular grouping which acted as a kind of nucleus to which other protein groups (for example those which were thermostable or thermolabile) could attach themselves. The mechanism of the energy exchange, which was characteristic of activity, was effected by a distinct vibratory movement of the whole or a definite part of the protoplasm. Owing to this specific oscillation, the protoplasm had the power of bringing about the breakdown of con-

tiguous food-stuffs. The "affinities" (specific oscillations) must be of a specific nature for each tissue and were probably somewhat akin to ferment action. Thus, in diabetes, the "affinities" which brought about the breakdown of carbohydrates were for some reason or another in a state of suspension, inoperative or actually destroyed, whereas those which dealt with the catabolism of fat were active. When the food-stuffs were brought into contact with the "affinities" an atomic re-arrangement, with entry of oxygen, occurred. The potential energy of the food-stuff now became available and caused a complete alteration in the "affinities"; an absorption of energy into the living substance took place at the moment of the catabolism of the food-stuff. The internal oscillations and changes in the cells, however, gradually used up all the energy, which was converted into heat and lost, and there was a return to the original condition, the "affinities" being again ready to begin work. The rate of the change depended on the nature of the living substance, the temperature, nervous influences and the conditions of the organism itself. He further maintained that the protein breakdown could have but one aim, viz. to destroy the protein-like nature of the material in order to free the organism from a substance for which it had but little use. The increase of living substance had nothing to do with the energy exchange; they were in Rubner's opinion quite separate functions, although the "affinities" connected with both series of changes were closely associated.

Speck.

Speck [1903] also expressed his belief that two forms of protein existed, but his idea of their subsequent fate was rather curious. The part of the protein of the food, which was not utilized for the formation of new tissue (living protein), was broken down into a nitrogen-containing part, which was rapidly converted into urea and a nitrogen-free rest, which was readily used as a source of energy. The tissue protein, after the death of the cell, was also converted into a nitrogen-containing and a nitrogen-free part, but the subsequent fate of these two parts was different. The nitrogen-free part under normal conditions was converted into a fat or carbohydrate-like substance and utilized for energy purposes. The nitrogen-containing part was not immediately converted into urea, but it formed a great variety of substances, which played an important part in metabolism and were eventually excreted as urea. He believed that oxygen deficiency played an all-important rôle in the breakdown of the tissue protein.

Folin.

Folin [1905] advanced an extremely interesting hypothesis which is essentially an interpretation of the so-called "laws" which govern the composition of the urine. He carried out a very elaborate series of analyses of normal urine obtained from subjects on standard diets, (1) rich in nitrogen, and (2) poor in nitrogen, both diets being practically free from purine, creatine and creatinine. As a result of his observations he maintained that there were two forms of catabolism which were essentially independent and quite different. "One kind is extremely variable in quantity, the other tends to remain constant. The one kind yields chiefly urea and inorganic sulphates, no creatinine and probably no neutral sulphur. The other, the constant catabolism, is largely represented by creatinine and neutral sulphur, and to a less extent by uric acid and ethereal sulphates. The more the total catabolism is reduced the more prominent become these representatives of the constant catabolism, the less prominent become the two chief representatives of the variable catabolism." To the constant type he gave the name of *tissue* or *endogenous* metabolism, and to the variable *intermediate* or *exogenous* metabolism. The following table of Folin's demonstrates his views very clearly:—

	Nitrogen-rich Diet.	Nitrogen-poor Diet.
Volume of urine	1170 c.c.	385 c.c.
Total nitrogen	16·8 gms.	3·60 gms.
Urea nitrogen	14·70 ,, =87·5 per cent.	2·20 ,, =61·7 per cent.
Ammonia nitrogen	0·49 ,, = 3·0 ,,	0·42 ,, =11·3 ,,
Uric acid nitrogen	0·18 ,, = 1·1 ,,	0·09 ,, = 2·5 ,,
Creatinine nitrogen	0·58 ,, = 3·6 ,,	0·60 ,, =17·2 ,,
Undetermined nitrogen	0·85 ,, = 4·9 ,,	0·27 ,, = 7·3 ,,
Total sulphur	3·64 ,,	0·76 ,,
Inorganic SO_3	3·27 ,, =90·0 ,,	0·46 ,, =60·5 ,,
Ethereal SO_3	0·19 ,, = 5·2 ,,	0·10 ,, =13·2 ,,
Neutral S	0·18 ,, = 4·8 ,,	0·20 ,, =26·3 ,,

Folin held that the arguments advanced for the immediate resynthesis hypothesis of absorbed protein were not valid, being based on purely teleological grounds. He considered that his observations necessarily led to the view that only a small amount of protein was required by the organism, namely, that necessary for the endogenous metabolism. He was further inclined to accept the evidence put forward by Chittenden as incontrovertible. He overlooked the essential fact regarding protein requirements that in all probability it is not so much the quantity as the quality of the food provided which is of importance, so long as we have not a perfect food supply, i.e. food which

will provide in exact amount the different materials requisite for protein tissue repair.

Folin's view as regards the two forms of metabolism has been generally accepted, although exception has been taken to some minor points. He is perhaps inclined to separate his endogenous and exogenous metabolism too completely; other workers have shown that although his products of endogenous metabolism are characteristic of this form, they are also found in the exogenous form. Noël Paton [1905] thought that some at least of Folin's results might be explained by variation in the activity of hepatic metabolism. Thus on a protein-poor diet the hepatic metabolism would be sluggish and must therefore fail to convert a large amount of the waste nitrogen into urea, while on a protein-rich diet with hepatic stimulation the conversion must be much more complete. He also thought that urea must be considered a definite end product of both endogenous and exogenous metabolism. Folin himself, however, is prepared to allow this, as he says that "the fact that the urea and inorganic sulphates represent chiefly the variable catabolism does of course not preclude the possibility that they also represent to some extent the constant catabolism".

The recent work of Pohl [1917] showed that uric acid might be raised to the level of creatinine as a representative of constant catabolism. The work of Denis and Minot [1917], and others, would suggest that if creatine and creatinine be related, the intake of large amounts of protein must influence in some way the metabolism of these substances. Indeed it is highly probable, in view of the large part played by the protein-rich muscle in metabolism, that marked alterations in protein intake, even if creatine and creatinine-free, would affect, if only to a slight extent, the output of products which are presumably closely associated with the metabolic changes in muscle.

CHAPTER VIII.

STARVATION.

MOST of the evidence offered up to this point in connexion with protein anabolism and catabolism has been drawn from experiments on feeding with individual amino acids, mixtures of these acids, single food-stuffs, or combinations of food-stuffs. But evidence of great value, particularly as regards the catabolism of protein, has been obtained by the investigation of the products excreted in the urine during complete or partial fasting. The value of this evidence has steadily increased, especially since the appearance of the work of Folin.

Output of Total Nitrogen.

Starvation experiments have been carried out both on men and animals. In the case of the men the majority of the fasters have been professional "Hungerkunstler". The metabolism of the best known of these "Hungerkunstler," Succi, has been investigated at least five times, by Luciani [1890], Lo Monaco [1894], Daiber [1896], E. and O. Freund [1901], and finally by Brugsch [1907]. Lehmann, Müller, Munk, Senator and Zuntz [1906] studied Cetti and Breithaupt. Hooven and Sollmann [1897] have investigated the metabolism of inanition in a man during hypnotic sleep. Complete studies of fasting metabolism with special reference to the nitrogenous waste products, in which the fasting period was preceded by a period of feeding on a definite diet, have been carried out on the professional faster, Beauté, by myself [1907], and on two non-professional fasters (university demonstrators) by Hawk and his pupils [1911]. Studies on fasting women have been carried out by Van Hoogenhuyze and Verploegh [1905], Bönniger and Mohr [1906], and by Benedict and Diefendorf [1907]. Benedict has published two volumes on metabolism during inanition. The one [1907] is mainly concerned with the energy output and carbon metabolism, but the other on subject L., who fasted for thirty-one days [1915], is the most complete and exhaustive study of inanition extant.

The longest fast recorded is that carried out by Howe, Matill and

Hawk [1912] on a dog, "Oscar". It lasted for 117 days, during which period the animal was given only water. In spite of the fact that its weight decreased from 26·33 kilos. to 9·76 kilos. a loss of 63 per cent., the animal was able to jump out of its cage quite actively on the 101st day. The total nitrogen output naturally fell, but the fall was somewhat irregular until the 81st day; thereafter it was regular until a slight rise occurred on the 117th day. It was not considered that this secondary rise was a premortal rise, as the creatine excretion did not exceed that of creatinine, a condition constantly observed with the premortal rise. The percentage urea output differed from human fasts (v.i.) in that no steady decrease was noted.

Starvation is characterized by a slow but steady fall in the output of nitrogen. The amount of the nitrogen excreted in the early days, as Voit showed years ago, is directly proportional to the amount of the previous nitrogen intake. A uniform output of nitrogen is practically always reached by the seventh day of the fast, irrespective of the nature or amount of the food taken before the fast. In practically every experiment the output of nitrogen is lower on the first and sometimes also the second day than during the two or three subsequent days. This is almost certainly due to the body utilizing its stores of free carbohydrate at this period, as Prauznitz [1892] in a long series of short experiments on students has clearly demonstrated. Benedict's work supports such an interpretation. It is interesting to note that in a series of short fasts on dogs, Underhill [1913, 2] found a tendency for the blood fat to diminish during the first three days of fasting and then to rise again even above the normal level. The blood sugar remained constant.

Benedict also made a careful study of the relation of the nitrogen output per kilo. body weight and found, as might have been expected, that it was very low on the first day of the fast, 0·118 gm. per kilo.—the lowest in the whole series. Thereafter it rose steadily until it reached a maximum on the fourth day of 0·207 gm., and this was followed in turn by a comparatively steady fall. As in my own case of Beauté, subsequent feeding with a low protein diet led to the output of about one-third of the quantity of nitrogen excreted on the last day of fasting.

The main interest, however, in these starvation studies when the organism is living on a purely endogenous supply of protein is not in the total nitrogen output but the partition of the nitrogenous constituents. See table which gives the results with Benedict's subject L.

PARTITION OF NITROGEN EXCRETED IN URINE IN EXPERIMENT WITH L.

Day of Fast.	Absolute Values in Gms.							Percentage Distribution (Per Cent. of T.N.).					
	T.N.	Urea N.	Ammonia N.	Uric Acid N.	Creatinine N (Preformed).	Creatinine N (Total).	Rest N.	Urea.	Ammonia.	Uric Acid.	Creatinine. (Preformed).	Creatinine (Total).	Rest.
Pre-day.	11·54	—	·59	—	—	—	—	—	—	—	—	—	—
1	7·10	5·68	0·41	0·112	0·51	0·48	0·42	80·00	5·77	1·58	7·18	6·76	5·89
2	8·40	6·69	·60	·049	·46	·46	·60	79·64	7·14	·58	5·48	5·48	7·16
3	11·34	9·11	·95	·042	·45	·55	·69	80·33	8·38	·37	4·06	4·85	6·07
4	11·87	9·03	1·40	·044	·42	·54	·86	76·07	11·79	·37	3·54	4·55	7·22
5	10·41	7·58	1·62	·059	·41	·51	·64	72·82	15·56	·57	3·94	4·90	6·15
10	10·05	7·44	1·59	·118	·37	·49	·41	74·03	15·82	1·17	3·68	4·88	4·10
15	8·46	6·18	1·45	·071	·30	·38	·38	73·05	17·14	·84	3·55	4·49	4·48
20	7·69	5·36	1·58	·115	·31	·38	·26	69·70	20·55	1·50	4·03	4·94	3·31
25	7·81	5·43	1·52	·098	·28	·35	·41	69·52	19·46	1·25	3·59	4·48	5·29
31	6·94	4·84	1·24	·122	·30	·32	·42	69·74	17·87	1·76	4·32	4·61	6·02

The Output of Urea and Ammonia.

Urea gradually falls both in relative and absolute amount. The same result has been observed by Folin and others when a subject is put upon a diet practically nitrogen-free. In the case of Beauté, during the fasting period the urea output fell to 71 per cent. of the total nitrogen, and that of L. to 66·67 per cent. on the nineteenth and to 69·74 on the thirty-first day. It must be noted, however, that the normal percentage output of urea in this subject was low. Hawk [1911] in his subjects found the lowest percentage output to be 75·8. E. and O. Freund [1901] observed on the twentieth day a much lower percentage output, viz. 56 per cent. Now it has been shown by Schroeder and many others that the main source of the urea is the ammonia which is formed in the body either from the denitrification of part of the ingested amino acids or from some other metabolic process taking place both in hunger and feeding. It is also a well-known fact that one of the symptoms of starvation is acidosis, as Brugsch [1905] in his investigations of Succi, and also Bönninger and Mohr [1906] have very clearly demonstrated. This acidosis is due presumably to the imperfect combustion of fat; under these circumstances, as one of the protective mechanisms of the body, the ammonia formed is used for the neutralization of the acids. The fall in the urea output is accordingly accompanied by a well-marked rise in the output of ammonia. In my investigation the percentage output of ammonia in relation to the total nitrogen rose from 3·16 per cent. on the last day of feeding to a maximum of 14·88 per cent. on the eighth day of the fast. The Freunds, although they obtained so conspicuous a fall

in the output of urea only observed a 2 per cent. increase in the ammonia output. If this figure be correct, it is extremely difficult to explain the enormous fall in the percentage output of urea which they found. Brugsch found in the urine of Succi on the twenty-ninth day of fasting a maximum ammonia output of 35·3 per cent. of the total nitrogen. Both Benedict and I noted in our subjects that there was a waxing and waning (which continued to the end of the fast in the case of L.) in the earlier part of the starvation period. Why the subsequent fall in the output of ammonia should take place it is rather difficult to say. One would have expected that as the organism became more dependent on its stores of fat and, as carbohydrate seems to be essential for the complete combustion of fat, there would have been a steady increase in the formation of organic acids and consequently an increase in ammonia required for neutralization. Such a steady rise in the output was not observed in L. The maximum output was on the nineteenth day.

The Output of Creatine and Creatinine.

The output of creatinine does not maintain the regularity which it exhibits under normal conditions. The investigations of Benedict and Diefendorf [1907], Van Hoogenhuyze and Verploegh [1905], Hawk [1911], Cathcart [1907], and Benedict [1907, 1915], show a decrease which is more marked in some experiments than in others. Thus in Beauté and in L. this fall was steady. In the case of L., from a maximum of 0·55 gm. total creatinine nitrogen on the third day to 0·32 gm. on the thirty-first day of the fast, whereas in the subjects of Hawk it was only slight. Accompanying the creatinine output there is in starvation an output of creatine (Cathcart, Benedict, Hawk, and others). In my subject the amount of creatine excreted first rose, then fell slightly, and subsequently remained more or less constant to the end of the fast. Hawk observed the same course in one of his subjects, whereas in the other the creatine excretion reached its maximum in the first day, and then steadily fell. Benedict noted in his experiment that the output of creatine gradually increased during the fast. If the combined output, creatinine and creatine, be considered, there is no marked fall, although the general tendency is towards a decrease. Hence the decreased creatinine output is compensated for by the output of creatine. Although the earlier figures cannot be wholly relied upon, owing to the fact that the aceto-acetic acid, which might have been present, was not removed previous to the determinations of the preformed creatinine thus rendering the creatine values too high, yet

sufficient evidence now exists to confirm the general statement that creatine is a constant constituent in the urine of starvation, the only uncertainty lies in, as Benedict remarks, "our knowledge of the quantities excreted".

From a study of the relationship of the output of creatine nitrogen to that of total nitrogen in the urine of the fasting bird, Noël Paton [1910] holds that light is thrown on the nature of the course of protein metabolism in the muscles and other organs. He thinks that if the muscle "flesh" catabolized, as calculated from the creatine excreted, be greater than the total "flesh" disintegrated, as calculated from the total nitrogen eliminated, the conclusion may be drawn that there has been a retention of some of the muscle protein nitrogen either due to resynthesis in the muscle or to transference to some more essential organ. On the other hand, when the "flesh" catabolized, calculated from the output of total nitrogen, exceeds that calculated from the output of creatine, the stored nitrogen, i.e. the circulating or surplus protein of Voit, has been broken down. He supports his hypothesis, by evidence obtained from geese fed in varying fashion before the fasting period. Thus, a fat young growing gander, abundantly fed previous to the fast of three days, catabolized during this time mainly non-muscle protein —i.e. the reserve protein had been adequate to tide the bird over the period of deprivation; a fully grown bird, which was fed on a comparatively nitrogen-poor diet previous to the three-day fast, had only a sufficient store of non-muscle protein for one day, and on the two subsequent days subsisted on muscle protein. Even when the reserve protein was all utilized and the bird was existing on its muscle protein there was a retention of part of this muscle nitrogen.

These facts are extremely interesting, particularly the latter with reference to the utilization of a stored protein, as it is further evidence in favour of the existence of a reserve store of protein for emergencies (see p. 93).

Noël Paton maintains that the same calculation can be applied in the case of human subjects, if the combined creatine and creatinine output be considered. Using the data of Van Hoogenhuyze and Verploegh, of Benedict and of Cathcart, on starving subjects, he finds that muscle flesh is the protein material utilized during the fast, and that as in the case of birds, there is a very well-defined retention of the muscle nitrogen as the fast progresses.

This alteration in the ratio of the output of total nitrogen to creatine which Noël Paton regards as indicative of the retention of muscle nitrogen is of considerable interest. One must infer that the creatine

or more probably creatine precursor is present in the muscle in a more or less labile form and that it is not directly required for the resynthetic processes. Urano [1906] showed that under certain conditions (*in vitro*) particularly if the tissue has undergone slight autolysis, creatine could be readily dialyzed out of muscle tissue. As creatine is present in considerable amount only in muscle tissue, and as muscle tissue, according to the observations of Voit and others, is much reduced during the course of starvation, it must be concluded that the liberated creatine is excreted and the creatine-free nitrogenous rest is utilized for the building up of tissues more immediately essential to the animal. Benedict has discussed the question as to whether creatinine output can be regarded as an index of the changes in the mass of active muscle tissue, but stated that his results showed no obvious relation between the total standard metabolism and the creatinine output. Palmer, Means and Gamble [1914], working with normal subjects, obtained similar results. It is interesting to note, however, that in subject L. the creatinine coefficient (i.e. total daily creatinine output divided by the body weight), which normally lies between 30 and 40 mgm., after the second day of fasting remained practically constant until the fourteenth day between 20 and 25 mgm. The mean output for the subsequent portion of the fasting period was about 19 mgm. daily.

Certain experiments which Hawk and Fowler [1910] carried out on the effect of water drinking must be considered here, as the results were interpreted by them as indicating the possibility of removing creatine from muscular tissue without any accompanying total catabolism of the muscle. Hawk [1911] in his fasting experiments found that less than 25 per cent of the total nitrogen output—i.e. protein catabolized—could be accounted for on the basis of the creatine eliminated. Thus in subject E. the amount of muscle protein catabolized calculated from the creatine output was equivalent to an output of only 20·06 gms. of nitrogen, whereas the actual output of total nitrogen was 86·44 gms. He and his co-workers have found that the creatine content of muscle may be decreased as much as 66 per cent. by fasting, with but slight reduction in the total nitrogen content. They concluded, therefore, that it may be logically inferred that part of the creatine had been removed from muscular tissue which was still functioning in the body. Mendel and Rose [1911, 2] found, however, that there was actually an increase in the creatine content of muscle during inanition, although Howe, Matill and Hawk [1912] stated definitely that the content in creatine was diminished.

In this connexion two observations of Abderhalden are of interest. Abderhalden, Bergell and Dörpinghaus [1904], on the one hand, could demonstrate no difference in chemical constitution between the tissues obtained from a normal and from a fasting animal. Abderhalden [1908, 4], on the other hand, found definite evidence of the retention of nitrogen in the tissues of an animal during fasting, the retention not being necessarily in the form of protein. He reached this conclusion from the observation that if a fasting animal were given a large amount of fluid there was a marked rise in the output of nitrogen. This rise in the output of nitrogen might, of course, be due to increased catabolism of protein due directly to the giving of the water, but Abderhalden considered such an explanation as highly improbable. In support of his conclusion he cited some previous work carried out by himself in conjunction with Bloch [1907] on an alkaptonuric patient. They gave a large quantity of water (5 litres) to the subject of the experiment who was on a fixed diet. The output of total nitrogen in the urine rose markedly but without any accompanying rise in the output of homogentisic acid. From this constancy in the homogentisic acid output Abderhalden concluded that the protein metabolism as such was quite uninfluenced by the giving of water, and that the increased output of total nitrogen was due simply to the washing out of "free" nitrogen—probably end products from the tissues. Grosser [1910] also, working with infants, found that the giving of water left the nitrogen metabolism practically unaffected. Hawk [1905], on the other hand, found that the giving of four and a half litres of water on two successive days to a man in nitrogenous equilibrium brought about a rise in the output of nitrogen in the urine on both days. As the output was greater on the first day than on the second day, Hawk thought there was a washing out of waste products from the tissues, and, at the same time, also an increase in the protein catabolism, as even on the second day there was an increased output of nitrogen. He produced further evidence in support of this contention in a later paper (Howe, Matill and Hawk [1911]) from experiments carried out on a dog. He found a rise on the first day but by the fourth day of the high intake the output was nearly normal. Orr [1914] found that the ingestion of large quantities of water accelerated both the catabolic and synthetic phases of protein metabolism.

In starvation particularly, it is almost certain that both sources of nitrogen must be considered. In all probability, in this condition much of the so-called "free" nitrogen is the result of autolytic action

in the tissues, and is material in transit for utilization. If this repair and fuel material be washed out, as life must be maintained, then there will be a further breakdown of protein to supply the deficiency. Probably the same hypothesis holds true in the fed animal provided the intake of nitrogen be not too large. Here by the excessive ingestion of water and resultant diuresis the absorbed material is washed away before it can be utilized. In order to supply the deficiency in the organism, which is now in a state of partial nitrogen starvation, there will be an increase in the catabolism of tissue protein. That some such balance as the one just presented does occur is borne out by the following observations. Straub [1899] was unable to get an increase in the output of nitrogen after two litres of water in a man in nitrogenous equilibrium on a large intake of protein, whereas Hawk [1905] on a diet containing half the amount of nitrogen did get an increase. Heilner [1906, 1907] was unable to get an increase in the output of nitrogen after giving water to well-fed dogs, although, on the other hand, he found 2000 c.c. of water given to a fasting dog increased the output of nitrogen and at the same time increased the output of chlorine by about 30 per cent. This increase, he maintained, was due not to mere washing out of the tissues but to increased catabolism of the protein. He based his conclusion on the fact that the increase in the output of chlorine was extended over several days whereas the nitrogen output rose at once with the increased diuresis. Trosianz [1911] also found that sodium chloride solution in varying strengths when injected into a fasting dog produced a definite rise in the output of nitrogen.

The loss of fluid from the body by hæmorrhage from injury also seems to be associated with an increase in the output of nitrogen (Hawk and Gies [1904], Haskins [1907], and Kerr, Hurwitz, and Whipple [1918, 1, 2, 3]). Fuchs [1909] found that even the loss of 35 per cent. to 58 per cent. of the original blood by hæmorrhage, where the fluid loss was replaced by isotonic saline, brought about no increase in the output but an actual retention of nitrogen. He did find, however [1910], that there was a very slight increase in the output of amino acids in the urine. Buell [1919], working with pigs, found after repeated bleeding that there was a rise in the output of total nitrogen and creatine. On the other hand, the natural hæmorrhage of menstruation is said to be accompanied by a slight but definite retention of nitrogen in the body (Schöndorff [1897] and Schrader [1894]).

The Output of Purines.

As regards the source of the purines it is evident that they may either arise from the breakdown of nucleoprotein in the tissues or by synthesis from non-purine material. There is a certain amount of evidence that synthesis is possible. Thus Cathcart [1909], Graham and Poulton [1913], and Umeda [1915] have all noted that a fat-rich protein-poor diet leads to a diminished output of purine, whereas a carbohydrate-rich one leads just as definitely to an increase in the purine output. Ackroyd and Hopkins [1916] found further that when arginine and histidine were removed from the diet a fall in the output of purine took place, and that when they were restored this was accompanied by a rise in the output. It would be natural to conclude that when these amino acids are liberated during starvation autolysis they might be synthetized to purines and thus increase the output. Lewis and Doisy [1918] also found that the administration of certain monamino acids like glycine and alanine could bring about a rise in the purine output. In view of the fact that the purine synthesis would seem to be associated in some way with the presence of an abundant supply of carbohydrate, a condition which is naturally lacking in starvation, it may be assumed that if a synthesis does take place during fasting it must only be to a limited extent.

The state of matters with reference to the preformed purine is not much clearer. Nemser [1899] definitely stated that the nuclein moiety of the tissue could resist longest disintegration in starvation. Such a view is highly probable because, the nucleoprotein being a component of the cell nucleus, it must be intimately, even if indirectly, connected with tissue regeneration. If this be true then it might be inferred that, as the fast progresses, there would be eventually a rise in the output of purine due to the catabolism of the precious nuclear material, i.e. when the organism could no longer obtain a supply of more available and less essential material. When the observed facts are considered it was found in the cases of Beauté [1907], L. [1915] and La Tosca [1905] that there is a fall in output of purine followed by a rise as the fast progresses. The deduction, then, that the body first utilizes in starvation tissues which, at the time, are of least value, or most readily replaced, preserving as long as possible those which are of greater importance, would appear to be valid.

Burian and Schur [1900] look upon the hypoxanthine of the muscle as the important source of urinary purine. It may be that this hypoxanthine is the sole or chief source during the early days of the fast,

but as the period of starvation lengthens other sources must be drawn upon. An attempt (Cathcart [1907]) was made to calculate from the total nitrogen output of Beauté the amount of endogenous purine which might be expected to be excreted.

The table shows a steady decrease in the calculated amount of purine nitrogen, whereas the columns giving the actual output of purine nitrogen show a steady and constant rise. From these figures it may be inferred either that tissues or parts of tissues rich in the precursors of the endogenous urinary purine are being increasingly

Day of Starvation.	Protein Tissue = Total Nitrogen × 6·25 × 5.	Calculated Yield of—		Actual Excretion of—	
		Purine Nitrogen = 0·03 Per Cent. (Muscle).	Purine Nitrogen = 0·06 Per Cent. (Muscle and Gland Tissue).	Uric Acid.	Total Purine.
1	328·5	0·098	0·197	0·12	0·15
2	449·4	0·134	0·269	0·06	0·11
3	428·8	0·128	0·256	0·06	0·09
4	428·8	0·128	0·256	0·08	0·14
5	353·1	0·106	0·212	—	—
6	336·5	0·100	0·200	0·10	0·14
7	302·2	0·090	0·180	0·12	0·15
8	297·5	0·089	0·178	0·12	0·15
9	293·4	0·088	0·176	—	—
10	261·8	0·078	0·156	0·16	0·20
11	265·3	0·079	0·158	0·16	0·20
12	274·0	0·082	0·164	0·17	0·19
13	280·3	0·084	0·168	—	—
14	243·1	0·073	0·146	0·17	(0·19 ?)

drawn upon as the fast progresses, or assuming the purine content of the tissues to be constant and a steady total catabolism of these tissues, that here also there is retention of part of the protein nitrogenous constituents as in the case of creatine.

The Output of Sulphur.

As stated already, the investigation of the sulphur output yields a certain amount of evidence as to the course of protein metabolism. It will be recalled that Von Wendt [1905] went so far as to maintain that it was only by considering the output of sulphur in relation to the output of nitrogen that a true idea of the course of protein metabolism could be obtained (p. 105). The course of sulphur output in starvation has been the subject of investigation on two or three occasions. As the ratio of nitrogen to sulphur in muscle is about 14 : 1 the figures obtained in starvation bear out the statement that a large part of the protein supply in this condition comes from muscle tissue. In my investigation the ratio of nitrogen to sulphur gradually fell during the

course of the fast from about 17 to 1 to 14·5 to 1. Halpern [1908] found at the end of a fast of twenty days a nitrogen sulphur ratio of 14·61 to 1, and finally Benedict with his subject L. found at first a slight increase to 17·7 on the fourth day of the fast, then a fairly steady fall to 14·2, the figure reached on the thirty-first day of starvation. There was a very close relationship noted between the output of nitrogen and sulphur. A slight but definite increase in the N : S ratio was noted on the twenty-fourth and twenty-fifth days of the fast, indicating presumably the sudden disintegration of some sulphur-poor material. Lewis [1916] in his experiments noted during the course of starvation a slight retention of sulphur. As the output of both creatinine (and creatine) and purine indicated the possibility of a certain retention of muscle tissue having taken place, it may be inferred that the partial splitting of the protein molecule which led to the liberation of these substances could not have proceeded so far as to set free the sulphur. On the other hand, it must be remembered that according to Ehrström, Falta, and others, the sulphur containing part of the molecule must be looked on as the labile part. It is, of course, possible that all the sulphur was liberated from the muscle protein, and that the balance of nitrogen excreted was made up from other sources. Wolf and Osterberg [1911, 2] inclined to the view that the sulphur-containing fraction of the protein molecule was of fundamental importance to the organism. Until, however, reliable analyses of tissues are available it is impossible to come to any definite conclusion. Voit and Korkunoff hold that the nitrogen excreted in hunger does not come from actual tissue protein alone, but may also originate from the extractives of the organs. They found that the tissue mass of a goose after five days' starvation contained 15·39 per cent. nitrogen. Of this 12·55 per cent. (81·55 per cent. of the total nitrogen) was in the form of protein, and 2·84 per cent. (18·45 per cent. of the total nitrogen) was of extractive nature.

The Output of Phosphorus.

Just as in the case of sulphur the output of phosphorus tends to decrease as the fast progresses. This fall is clearly indicated both in the observations on Beauté and on L. The N : P_2O_5 ratio in the case of Beauté reached a maximum of 6·22 on the fourteenth (last) day of the fast whereas in L. the maximum of 5·96 was reached on the twelfth day; thereafter the ratio remained fairly constant with an average value of just over five. The theoretical value for muscle is about 6·6. Benedict pointed out that when the outputs of phosphorus

are considered, there seemed to be "a tendency towards a much larger excretion of phosphorus pentoxide in its relation to nitrogen than occurs in the ordinary composition of flesh". This conclusion is almost certainly correct because in all probability the bony tissue contributes materially to the output of phosphorus; there is very general agreement on this point. During his thirty-one days' fast L. excreted 277·32 gms. nitrogen corresponding to 8319·6 gms. protein calculated as flesh. Assuming that this protein had normally combined with it 0·5 per cent. phosphorus pentoxide it would mean that there should have been some 41·6 gms. P_2O_5 liberated whereas there was actually a total output of 56·36 gms. during the fast, i.e. assuming the correctness of the estimate of phosphorus in flesh, there was an excess excretion of some 15 gms. which had come from some other source. The disintegration of phosphorus rich nucleoprotein, as pointed out in the discussion on purine output, may also account in part for the $N : P_2O_5$ ratio in inanition.

The Capacity of the Starving Organism to deal with Injected Amino Acids.

Some modern work has also been carried out on the power possessed by the fasting animal of retaining and breaking down amino acids. Thus Hirsch [1905] finds that the organism deals as usual with amino acids after injection into the tissues. Later Brugsch and Hirsch [1906], working with a professional faster, found, so far as they could judge, no increase in the amino acid excretion during hunger even after the administration of comparatively large amounts of amino acids; on the contrary a retention of nitrogen might even occur when amino acids were injected.

Autolysis.

It is evident then that, although the supply of protein is cut off in starvation, the demand for nitrogenous material by certain essential organs still goes on. As there is no excessive reserve of store protein which can be drawn upon, the organism must meet the demand by the sacrifice of its own less essential tissues. This solution or disintegration of body tissue is known as autolysis and it is brought about owing to the fact that each tissue contains proteolytic enzymes capable of producing a disintegration of the protein tissue apparently similar in every way to that produced by the action of the ordinary proteolytic enzymes like trypsin. It is more than probable that these enzymes play an active part during the normal life of the organism that they are

proteosynthetic as well as proteoclastic. For such a conception there are many analogies; take, for example, the proved reversible action of maltase and of lipase, the work of Robertson (l.c.) and Taylor (l.c.) on pepsin and trypsin, and the probable reversible action which goes on in the liver, the conversion of sugar into glycogen, and of glycogen back into sugar. Knoop [1910] has also shown that the first phases of the oxydative breakdown of amino acids in the tissues are reversible. What are the peculiar conditions, then, under which this catabolic function gains the upper hand in starvation? Is it because the constitution and amount of the blood proteins are interfered with? Burckhardt [1883], Lewinski [1903], Gitken [1904], Inagaki [1907] have all found a reduction in the amount of protein present in the blood in fasting, the decrease being mainly in the albumin fraction, although Kerr, Hurwitz, and Whipple [1918, 1, 2, 3] have found that fasting influenced but little the protein content of serum. Hedin [1904] and Cathcart [1904] have both shown that the serum possesses active anti-proteolytic properties and that this anti-proteolytic action is associated with the albumin fraction; thus it may be that the increased autolysis in fasting is due in part to the disappearance of the serum albumin. Schryver, on the other hand [1906], holds that the autolytic enzymes are held in check by purely chemical means. In a series of experiments he demonstrated that a diminution of the alkalinity of the tissues and fluids of the body from any cause was followed by an increase in proteolytic activity. He found that acids, especially lactic acid, accelerated the process of cellular disintegration. The formation of acid, and particularly of lactic acid, is one of the constant phenomena in autolysis (Lindemann [1911]). Mochizuki and Arima [1906], Inouye and Kondo [1907], Frew [1909], Turkel [1909], Arinkin [1907] have all come to the conclusion that both organic and inorganic acids increase the extent of autolysis in the liver. Schryver believes that the ammonia arising from the breakdown of protein is largely responsible for maintaining the protective alkalinity. The normal blood serum is not only resistant to the action of trypsin, but is capable of inhibiting the normal action of this enzyme on other proteins which are present, and which would be fully digested in the ordinary course of events. Oppenheimer and Aron [1904] hold, however, that something more than the mere presence of an "anti-trypsin" is involved in this resistance of normal serum. In support of this contention they quote the observations of Schwarz [1901]. This worker held that protein was resistant because of a peculiar combination of aldehyde groups in the protein molecule which offered but few points of attack for the

enzyme. Fischer has also shown in another connexion that a certain degree of specificity in formation of the substrate can exist, and of course it is possible that such an explanation may be the correct one here. It may be that the proteins under certain unnatural conditions become so altered that they are open to the attack of enzymes to which at other times they are immune. Until, however, our knowledge of the nature and constitution of the protein molecule is more intimate it is useless to speculate with any degree of certainty along such lines as these.

Some very interesting experiments have been carried out in the autolysis of yeasts and moulds. Dox [1913] has shown with *aspergillus niger* that the mould grew readily and well, utilizing nitrogen freely, on a sucrose medium, but as soon as the sugar was exhausted autolysis set in. Lampitt [1919] has also shown that in the case of *saccharomyces cerevisiae* the supply and concentration of sugar in the medium plays a most important part in the taking up and the giving off of nitrogen. Apparently then the regulation of the activity of the endocellular enzymes which are responsible for autolysis depend very largely on the state of nutrition of the cell and the availability of the food supply.

As regards the presence of the proteolytic enzymes in tissues, it was first pointed out by Salkowski [1890] that fresh tissues kept (antiseptically) at body temperature slowly dissolved, and the protein was replaced by various amino acids. To this change he gave the name of auto-digestion. Jacoby [1900] carried these investigations further. He showed that a variety of the known decomposition products of protein could be detected in the liquefied tissue and that the change took place much more rapidly under aseptic conditions. He gave the name of autolysis to the process of disintegration. Hedin and Rowland [1901] and Hedin [1906] were able to demonstrate the presence of proteolytic enzymes in juices expressed under high pressure from various tissues and organs. Further, Hedin found more than one form of autolytic enzyme in the spleen. Vernon [1910] has also proved the presence of an erepsin-like ferment in the tissues. Finally Leathes [1902], Dakin [1904], and Cathcart [1905], using modern methods, have shown that the various amino acids formed in the process of autolysis are apparently identical with those produced by the action of the true digestive ferments.

Abderhalden and Prym [1907] have demonstrated that the liberation of the different amino acids during the progress of autolysis is only gradual; even after fifty days' autolysis a fair amount of the products are present in a more complex form. Abderhalden and Lussana

[1908] have further shown that the expressed juices from different tissues can decompose various polypeptides. Abderhalden and his pupils [1909] introduced polariscopic methods for the investigation of the intracellular or other ferments found in the plasma. They maintained that when the occasion arose the organism could secrete into the plasma a ferment or ferments to deal with products which were not normally present in this fluid. A long series of observations were made after the subcutaneous or intravenous injection of various proteins. It was invariably found that a ferment was formed, which was not specific for any definite type of protein. Moreover, if the protein, raw egg albumin, for example, were taken in large amount by the mouth, the part which, as already mentioned (see p. 11), was absorbed unchanged, generated a specific ferment in the plasma. Zunz [1912] has definitely stated that the normal serum if kept at 38° shows proteolytic activity towards its own proteins, an activity which was more marked when serum proteins of another species were acted upon. He believed that normal serum contained some anti-body which kept the proteolytic enzyme in check.

CHAPTER IX.

WORK.

The Influence of Work on the Output of Nitrogen.

As the endogenous output of nitrogen during rest is so small it might naturally be expected that it would be increased when the conditions were altered, i.e. when more active metabolism took place as during work. Practically every investigator of this problem has found that work leads to little change in the output of nitrogen in the urine, provided always that the supply of food, particularly of carbohydrate and of oxygen, be sufficient. Gerhartz [1910] indeed inclines to the view that the metabolism generally is reduced by work. He found that the output of all the mineral constituents with the exception of sodium was reduced. The source of energy for the performance of work has been the subject of investigation for a great many years, and naturally the most divergent views are found. Thus, Voit and his school looked on fat as the most important source of energy; Chauveau and Seegen considered that carbohydrate was the important material; Pflüger at first held that the sole source of energy lay in the protein, although later he modified the idea to some extent. Others held that all three foods played a part in the supply of energy.

No one has yet been able to show clearly that during work there is any great utilization of protein, as evidenced by a marked increase in the output of nitrogenous waste products in the urine. Thus Voit [1881] found that if a fasting animal were exercised there was only a small increase in the output of nitrogen. In a thin young animal the increase varied from 8 to 16 per cent. of the total amount, and in an old fat animal, after eight hours of hard continuous work, the increase was from 1 to 8 per cent. of the total amount of nitrogen excreted. The differences noticed between the effect of exercise taken on a full or an empty stomach were only slight. Voit and Pettenkofer [1881] confirmed on a man this negative effect on the nitrogen output during work carried out both in periods of starvation and feeding. Voit was inclined to ascribe the slight rises observed after work to the complete utilization of the nitrogen-free food-stuffs being followed

by the burning up of some of the protein tissue, owing to the lack of fuel. Oppenheim [1880], who only found an increase in the nitrogen output after work when dyspnœa was induced, regarded the rise as secondary, and not as the direct result of an increased catabolism of protein due to the actual work. Fränkel [1876], [1877] obtained the same result and ascribed it simply to the lack of oxygen. Voit [1907] also observed this rise of nitrogen excretion after dyspnœa, but thought that the muscular work, arising from the struggle for breath, utilized all the available nitrogen-free material, and then drew upon the tissue protein. Argutinsky [1890] observed a rise in the output of nitrogen, but not until some three days after the work had been carried out. Liebig previously had suggested that the rise in the output of nitrogen would not be observed on the day of work but later. This is apparently due to the fact that a certain amount of damage is done to the cells by work, and that cell restitution with the coincident excretion of effete material is not a sudden act, but a comparatively slow one. Zuntz [1894], Munk [1890], and Kaup [1902], all strongly supported the view that work was followed by a rise in the output of nitrogen only when there was an inadequate supply of nitrogen-free food material available. Hirschfeld [1889], [1890] found an increase in the nitrogen output following work, only if the diet were deficient in amount. Unfortunately Hirschfeld did not devote much care to the nitrogen analysis of his food-stuffs and therefore his results cannot be regarded as conclusive.

Pflüger [1891] fed a dog of about 30 kilos. weight, doing severe work, for some seven and a half months on flesh, which contained only a mere trace of fat and sugar. He concluded that protein alone was sufficient to supply all the necessary energy, and that indeed protein was the food par excellence—fat and carbohydrate would only be utilized when all protein supplies failed. In this animal a slight rise in the output of nitrogen was always observed after work, but it was certainly not commensurate with the amount of work done. Further, the rise, such as it was, did not take place on the day of work, but on the second and third days following the exercise. In his conclusion Pflüger stated definitely that there was no work without some increase in the catabolism of protein as an accompaniment. Pflüger [1903] admitted later that non-nitrogenous food might play some part in contributing energy for muscle work, but he still believed that the nitrogen-containing material played the really important part. In support of the view that the non-nitrogenous food material is utilized during work are the interesting experiments of Hohlweg [1911]. This

worker caused a dog to run on a treadmill and injected into it subcutaneously various sugars. He found that, when compared with the results of similar experiments made on the dog at rest, there was a marked reduction in the output of sugar in the urine after galactose and maltose, whereas with the disaccharides, sucrose and lactose, the former was utilized to a small extent and the latter apparently not at all.

Noël Paton and others [1897] found that after moderate work the rise in the output of nitrogen was small, but after excessive work, there was a marked rise in the nitrogen excretion. This marked rise might have been due to the complete utilization of the non-nitrogenous food material bringing about the breakdown of protein. Krummacher [1896] found that there was an increase in the output of nitrogen in a man doing measured work, and that the increased output took place even when a very large amount of protein was ingested. Further, he showed that the possible yield of energy, calculated from the nitrogen excreted, did not equal the energy expended in the work. Frentzel [1897] likewise demonstrated that, even if the total nitrogen excreted on the day of work, and not only the excess of nitrogen excreted, were regarded as coming from protein utilized during work, the material utilized would not be sufficient to furnish the energy expended. In one experiment the amount of nitrogen excreted accounted for only about two-thirds of the energy expended. Zuntz and Schumburg [1901] found in marching that the increase in nitrogen excretion took place two or three days after the work. They also noted that other factors besides the actual work influenced this output of nitrogen—that the amount of work, and the degree of protein catabolism, did not run exactly parallel. Thus a much greater excretion of nitrogen followed a march with a light load on a warm day than with a full load and a normal temperature. Caspari [1901] alternately rested and worked dogs in nitrogenous equilibrium and found a slight increase in the nitrogen output after work. Even this small rise he was inclined to ascribe to faulty dietetic conditions—not to the supply of the food being insufficient to cover the energy expended.

Shaffer [1908] carried out a series of experiments in which the effect of increased and decreased muscular activity was tested. He found that with a sufficient supply of food work had no effect on catabolism as indicated by the nitrogen and sulphur excretion in the urine. The creatinine output was also quite unaffected, although it is generally held that there is some close relationship between the creatinine output and the amount of active muscular tissue in the body. Garratt [1898]

found that as the result of exercise there was a slight increase in the output of nitrogen. He observed that the rise in the nitrogen output was preceded by a rise in the output of sulphur. Engelmann [1871] also found an early rise in the output of sulphur as the result of exercise.

Frank and Gebhard [1902] attacked the problem in another way. They argued that if the muscular metabolism were reduced below normal by the injection of curare, the endogenous nitrogen exchange ought to be influenced. Accordingly they curarized a dog and found as they surmised that the nitrogen output was reduced about 25 per cent. They held that their experiments demonstrated that a certain preparation or storing of material took place, so that the organism was always ready for activity, but their argument is extremely difficult to follow. Frank and Voit [1901] had previously shown that the carbon exchange was hardly altered by the giving of curare.

Difference between Voluntary and Involuntary Muscle Contraction.

There would seem to be the possibility of two different forms of muscle activity which affect the metabolism differently—the one associated with the voluntary contraction and the other with the involuntary. Leathes and Cathcart [1907] demonstrated that the whole course of purine excretion can be modified by alterations in the intensity of the involuntary work. In these experiments shivering was utilized as the form of involuntary work. It was found that if a subject, doing the minimum amount of voluntary work, were exposed to cold, so that severe shivering was induced, a very marked rise in the output of uric acid followed, whereas when involuntary work was reduced to a minimum and voluntary work carried out even to excess, there was just as marked a fall in the output of uric acid. In both sets of experiments the food was identical, sufficient in amount, and purine-free.

Another interesting point of difference in muscular metabolism which has been pointed out by Graham Brown and Cathcart [1909] and by Pekelharing and Van Hoogenhuyze [1909], is that white muscle always contains more creatine than red muscle. The latter observers calculate that there is at least one-fourth more creatine present in white muscle than in red. Pekelharing and Harkink [1911] found that the creatinine output was quite unaffected by muscle work but that after prolonged tonic contraction the output rose. They held that definite evidence existed for a differentiation in the chemical changes during muscle tonus and work.

Very definite differences exist, as Buglia and Costantino [1912] have pointed out, between the chemical composition of the various types of muscle, striated, cardiac and smooth. They found that striated muscle was richer in total nitrogen, amino nitrogen, total creatinine and carnosine nitrogen than the other two, and that although smooth muscle contained more total and amino nitrogen than heart muscle it was poorer in total creatinine, carnosine and purine nitrogen. Heart muscle contained more purine nitrogen than striated muscle.

The Influence of Work on General Metabolism.

Bornstein [1901] maintained that work plays some part in the general metabolism of protein, even if it have no marked action on the catabolism of this substance. He found that there was quite a marked retention of nitrogen if protein were fed during the period in which work was being carried out. He held that the nitrogenous metabolism, on a constant protein intake, was alike both for rest and work, but that there was a difference in the nature of the protein consumed in the two conditions—a difference in the amount of tissue and circulating protein utilized in the nomenclature of Voit (or in the amount of old and fresh organized protein in that of Pflüger). There might be an increase in the amount of old organized protein broken down, but so long as there was a supply of material available for the formation of the new protein, there would be no variation in the output of nitrogen. Hypertrophy of muscle due to activity takes place more freely when the amount of material present for building purposes is large. Von Noorden believes that part of the retention even under these conditions may be in the form of an inert reserve protein. Atkinson [1918], however, came to the conclusion that mechanical work had no influence on either the hourly rate of absorption of protein or on the intensity of the hourly metabolism of protein in the case of a dog which had been given a large meat meal before the work started.

Why does Work have so Little Apparent Influence on the Catabolism of Protein?

What explanation is to be offered for the apparent anomaly that work, which presumably increases, as evidenced by the greatly increased intake of oxygen and output of carbon dioxide, the metabolism of the tissues, mainly composed of protein, is accompanied by but little increase of nitrogenous waste products in the urine? Examination of the muscle itself has shown certain differences after

work. Thus Burian [1905] and McLeod [1899] have shown that the purine content is altered, Brown and Cathcart [1909] have shown that there is an alteration in the creatine content, and Pekelharing and Van Hoogenhuyze [1909] have found that a chemical change takes place after stimulation and in rigor mortis which leads to the formation of creatine in muscle tissue. Pugliese [1911] found, however, in his experiments that work altered but little the nitrogen content of muscle, although there was a slight alteration in the amount of the non-coagulable nitrogen of the blood. Feigl [1916] also found but slight alteration in the blood as the result of exercise.

It is a fundamental law in mechanics that every piece of machinery wears with work and that the greatest amount of wear takes place in those parts which are most frequently in use, and where friction is greatest. It is a point of very considerable importance if the tissues, which are most deeply involved both at rest and at work—muscular tissue in particular—do not obey this law. Are they to be considered as practically unwearable, like the jewelled bearing of a watch? Such a conception can hardly be true, for, if the work be carried out under unfavourable conditions, there is soon evidence of use in the increased output of nitrogen in the urine. Why then, under absolutely normal conditions, is there little or no evidence of use? Either (1) the actual wastage of protein is small in amount; the protein tissue as it is broken down separates into two distinct portions, one of which, the non-nitrogenous part, is used solely for dynamic purposes, whereas the nitrogen-containing moiety is reutilized—resynthetized—within the body; or (2) the protein tissue is actually broken down, but an equivalent amount of nitrogen is taken from the food supply to replace that wasted, with the result that there is little increase in the amount of nitrogen excreted.

It is well-nigh impossible to state which of these two hypotheses approximates more closely to the true condition. The first presupposes only a small requirement of protein for the body. It fits in extremely well with the facts observed in connexion with the output of endogenous nitrogen waste products, and in those feeding experiments where the daily intake of protein is small. The small increase in the output of nitrogen during work, and the apparently small endogenous exchange do apparently balance one another.

The second hypothesis has little or no experimental evidence to support it. It seems to me it would practically entail the acceptance of Pflüger's statement that the body needs are satisfied mainly by protein. As already pointed out, there is direct evidence that the energy

needs of the body are not solely supplied from a protein source during work. It would also involve the acceptance of the statement that the amount of nitrogen excreted represents exactly the amount of protein catabolism which has taken place in the tissues. Such a belief cannot now be adhered to. Evidence is steadily growing which shows that *all* the nitrogen ingested is not necessarily converted into urea with subsequent rapid excretion, but that part of it may be retained in the body either by resynthesis into fresh tissue protein or some simple form not yet definitely identified.

Although resynthesis certainly takes place, still every case of nitrogen retention cannot be attributed to it without further evidence. (See for example, p. 100.) Resynthesis of the nitrogen-containing part of the protein which is presumably catabolized during work is, however, extremely probable. Such a reutilization of the tissue nitrogen must and does take place during starvation as certain tissues and organs, even when doing steady work, as the heart, for example, retain practically their original weight up to the last (Voit, Chossat). This idea of a resynthesis of the catabolized nitrogenous material taking place within the tissues, particularly the muscles, is by no means a new one. Hermann [1867] put forward the hypothesis that the protein in all probability was decomposed into a nitrogen-containing part which was reutilized in some way, and a nitrogen-free part which was burnt. He believed further that an increased output of nitrogen took place only when the work done was very prolonged and severe —when an actual destruction of the muscle fibres was brought about. Pflüger [1891] has also suggested that the protein molecule might break down into two distinct parts, one containing the nitrogen which could be reutilized for the formation of new protein, and the other nitrogen-free which could be used to satisfy the dynamic needs. Verworn (" Textbook of General Physiology") supports such a view, believing that under certain circumstances regeneration of the nitrogenous residues can take place at the expense of other food-stuffs and oxygen. He maintains that this economical use of the costly nitrogen is wholly in accord with the other economies of nature. Cathcart [1909] also found that creatine, a substance which is not present under normal conditions in the urine, always appeared (see p. 117) during starvation. It was assumed that the appearance of this substance was due to the absence from the tissues of some material which directly or indirectly caused its retention. Investigation showed that the creatine present in the urine as the result of a fast always disappeared if carbohydrate food were given, but not if either protein or fats were given.

Fats given for several days, however, may bring about the disappearance of the creatine from the urine of a dog or at least a reduction in the amount present, but, so far, this has not been observed in man. It is probable that the canine metabolism as regards fats is more adaptable than the human. Wolf and Osterberg [1911] were unable to cause the disappearance of creatine by means of fat administration although both protein and carbohydrate were effective. Pari [1908] has also obtained certain experimental data from feeding dogs with fat after starvation which might also be interpreted as evidence of the possibility of adaptation taking place. Mendel and Rose [1911, 1] confirmed the observation that the giving of carbohydrate checked the output of creatine although they did not accept Cathcart's interpretation (v.i.), and other workers, notably Benedict and Osterberg [1914], Underhill and Baumann [1916, 1], Orr [1918], and Meyer have extended the work.

Cathcart, however, was of the opinion that his experiments supported the view that the reutilization of nitrogenous material, set free by the disintegration of tissue protein, in which carbohydrate played an important part was a normal process. As regards the form in which the nitrogen is reutilized no definite evidence is available; in all probability it is a very complex molecule containing creatine or more probably a creatine precursor as a labile constituent. Folin and Denis [1914] have stated that muscle creatine is a post-mortem product pure and simple, and others (Towles and Voegtlin [1911]) have suggested that neither creatine nor creatinine are true end products of protein metabolism, or that (Benedict and Osterberg [1914]) the creatine excreted does not necessarily arise from preformed creatine in the muscle: it may be synthetized. It is not proposed to develop this particular theme in the present volume as a special monograph dealing with the metabolism of creatine and creatinine is projected.

CHAPTER X.

INFLUENCE OF CARBOHYDRATES AND FATS ON PROTEIN METABOLISM.

THROUGHOUT the previous pages the question of the part played by the non-nitrogenous moiety of the diet in the anabolism and catabolism of protein has been incidentally referred to. In the present chapter more direct evidence is brought forward to emphasize the point that although a supply of protein is absolutely essential for the well-being of the organism, the other food materials do more than merely furnish a supply of energy.

Of the two great classes of non-nitrogenous material, so far as our present knowledge goes, it would appear that the carbohydrate is the more important. Indeed many workers take the extreme attitude that although the presence of fat in a dietary may be desirable it is not essential. Thus Von Pirquet [1917] states definitely that a fat minimum does not exist, that fat can be completely replaced by carbohydrate in the diet. He is supported by Gröer [1919] who brings forward, however, no new evidence. The contrary view that fat is also essential though perhaps in small amount is, in my opinion, the more correct view. Rubner [1919] in a recent paper has adopted the same stand, maintaining that fat cannot be completely removed from a diet. He maintains that tissue fat does not behave in the same way as fat given as food. Aron [1918] has also shown that fats cannot be wholly replaced by carbohydrates. He found that one series of animals who were allowed to eat *ad libitum* of a fat-free diet died, whereas a similar series on the same diet to which an addition of about 2·5 per cent. of butter was added lived. See also the long series of experiments carried out by Stepp in which he claimed that the bad results of fat-free food were not wholly due to the lack of accessory substances (p. 89), and the recent work of Mendel and others in connexion with accessory substances.

The Part Played by Carbohydrates.

That carbohydrates play an exceptionally important part in the utilization of protein generally has been repeatedly demonstrated.

The best example of this is found in the feeding experiments with abiuret digestion products where, unless there be an abundant supply of carbohydrate, there is no retention of nitrogen. The experiments of Lesser (see p. 38), who attempted to repeat the work of Loewi, give a most excellent demonstration of this fact. It will be remembered that this worker was unable to confirm the findings of Loewi, but an examination of his protocols showed that he used fats only to make up the caloric deficiencies of his dietary, omitting carbohydrate completely. So far as I am aware Abderhalden, Messner and Windrath [1909] are the only workers who have ever offered experimental evidence of a retention of nitrogen without carbohydrate being also present in the diet. As has been already suggested (p. 43) this result is in all probability due to the fact that the necessary carbohydrate was obtained from the protein or the fat in the diet. Lüthje [1906] has also clearly demonstrated in his feeding experiments on rabbits that the carbohydrate moiety of the diet is of absolute importance in the utilization of protein. He suggested that some form of amino sugar was first formed. Ross Taylor and Cathcart [1910] have shown that if glycosuria be induced by injections of phloridzin, there is an immediate appearance of creatine in the urine, which only lasts as long as the glycosuria exists, thus confirming the previous observations on the close relationship between the output of creatine and carbohydrate metabolism. These observations have been fully confirmed and extended by Krause and Cramer [1910], and by Wolf [1911], McAdam [1915], Tsuji [1915, 2], and Underhill and Baumann [1916, 1]. The appearance of creatine under these conditions is to be regarded as an index of faulty metabolism in general.

Falta, Grote and Staehelin [1907] found that when the metabolism of carbohydrate was interfered with by the removal of the pancreas, there was a marked rise in the breakdown of body protein as evidenced by the increased output of nitrogen. They hold that carbohydrate is essential for the general metabolic processes of the body, and if the animal cannot get it directly, then it obtains it indirectly from the protein; the sudden increase in the output of nitrogen is thus accounted for.

Why should carbohydrate be continually produced in these cases of experimental diabetes even to the extent of breaking down protein tissue after all the free sugar has been excreted? It is hardly probable that it is formed merely to be turned out again—a mere disturbance in the normal mode of catabolism. It must be produced as the result of a definite call of the cells for carbohydrate—a substance essential

to their very existence. The fact that the last traces of glycogen in hunger disappear but slowly, more especially from the muscles, and that sugar, although it is so readily utilized, is never absent from the blood, even at the end of starvation, is alone sufficient to demonstrate the vital importance of carbohydrate. It is probably a provision for the proper reutilization of the products of protein disintegration which arise from the autolysis of the tissues during this condition and which are required as food-stuffs by the heart and other essential organs. Falta and Gigon [1908] carried out a series of experiments on the utilization of proteins in the presence of carbohydrates and fats, and they also reached the conclusion that carbohydrate was absolutely essential to the animal organism. They state definitely that retention of nitrogen only takes place in the presence of carbohydrates. Shaffer and Coleman [1909] have clearly demonstrated that the "toxic" destruction of body protein in fever may be largely prevented by the intake of carbohydrate.

The observations of the botanical physiologists also support such a contention. Hansteen [1899], Ivanoff, and others, have repeatedly demonstrated that the presence of carbohydrate is essential before protein synthesis can take place in plants. Ivanoff [1906] found that the synthesis of the organic phosphorus compounds in yeast did not take place in the absence of the decomposition products of sugar which are formed during alcoholic fermentation. The phosphoric acid is supposed by him to combine with an aldehyde-ketone-group. Czapek [1901] found that moulds did not grow well in a medium containing an abundant supply of amino acids or other suitable nitrogen compound if carbohydrate were absent. He found that the carbohydrate which was most readily utilized was glucose. Kinoshita [1895] and Suzuki [1897] carried out a series of experiments in plants and concluded that ammonia could be taken up and be synthetized to asparagine if a sufficiency of carbohydrates were present.

In this connexion the fact must not be overlooked that there are present in nature various compounds of carbohydrate and nitrogen like the glucoproteins, and the very interesting substance glucosamine which is probably closely connected with the widely distributed group of betaines. (See Armstrong, "The Simple Carbohydrates and the Glucosides," London, 1919.)

Experimental Data.

(*a*) *In vitro* Experiments.

Until comparatively recently no experimental work on the possible nature of this intracellular synthesis existed. Pflüger has suggested that the part of the disintegrated protein molecule which was nitrogen-rich combined with "alcohol radicles" to form new protein. There is much *in vitro* work which offers valuable suggestions as to the probable nature of the processes which occur. It has long been well known that aldehydes form compounds with many different substances containing nitrogen, e.g. aldehyde ammonia. Sugars have also been shown to react like the simple acetaldehyde; Lobry de Bruyn [1894] made compounds of sugar with ammonia. Other nitrogenous substances more complex than ammonia have also been shown to unite with different aldehydes. Morrell and Bellars [1907] have obtained a definite compound of glucose and guanidine, and Wolfe [1894] has prepared the compound with aminoguanidine. Schoorl [1900] has made a compound of glucose and urea, and Jaffe [1902] a compound of creatine or creatinine and formaldehyde. Sörensen [1907] has shown that the simple amino acids react with formaldehyde with the formation of methylene compounds. Irvine [1909] carried out some very interesting work on the nature of the condensation which took place between sugars and amino bases. He found that in the case of glucose anilide it was not the simple aldehyde condensation which occurred as was formerly believed. Spiegel [1905] suggested that certain polypeptide groups present in the protein molecule were linked together by means of carbon atoms, and he attempted to bring about a synthesis of protein from protein decomposition products and formaldehyde. He stated that he obtained products which suggested that a synthetic change had taken place.

It is more than probable that it is the union of the nitrogen-containing radicles with the reactive aldehyde or ketone groups which brings about the protein synthesis in the body. Very reactive aldehydes and ketones such as methylglyoxal and glyceric aldehyde can certainly be formed in the decomposition of the carbohydrates, and indeed they probably arise during the normal course of carbohydrate catabolism in the organism; at any rate various products known to arise during the decomposition of carbohydrates *in vitro* have also been recovered from animal tissues. The very suggestive paper by Dakin and Dudley [1913, 1] on the dissociation of amino acids in dilute solution also demonstrates the formation of similar highly

reactive substances from the non-nitrogenous moiety of the protein molecule (see p. 54).

As Bondi [1909], Bondi and Frankl [1909], and Abderhalden and Funk [1910] have shown it is also possible to obtain compounds of proteins and fats—the so-called lipo-peptides or lipo-proteins. A large number of compounds have been prepared from palmitin and stearin with glycine, alanine, tyrosine, phenyl alanine, leucine, cystine glutamic acid and tryptophan. Abderhalden and Guggenheim [1910] also managed to obtain a compound of tyrosine with glycerine, and Abderhalden and Kautsch [1910] a good yield of glycyl cholesterol.

(*b*) *In vivo* Experiments.

A large amount of experimental work has been carried out *in vivo*. Thus Spiro [1907] found that, if glycine and fructose were injected into an animal simultaneously, a dicarbonic acid could be isolated from the urine which was not present when either substance was injected alone. Knoop [1910] made the first real advance in this field. He found that, after the injection of an α-keto acid, nitrogen could be added on and an α-amino acid formed within the tissues. The synthetized substance was asymmetric. It would thus appear that the first phase of the oxydative breakdown of the amino acid is a reversible process. He also found that α-oxy acids could be converted into α-amino acids. He held that this proved the possibility of the union of the decomposition products of sugar and ammonia to form a protein nucleus. Knoop and Kertess [1911] have fully confirmed this previous finding. Dakin and Dudley [1914] were able definitely to demonstrate the formation of leucine, α-amino-phenylacetic and possibly of phenylalanine from isobutyl, phenyl and benzyl glyoxals. They possibly obtained small amounts of glycine from glyoxal but certainly no alanine from methyl glyoxal. Embden and Schmitz [1910], [1911] attacked the problem in another fashion and have contributed valuable confirmatory evidence. These workers perfused the glycogen-poor liver with blood to which had been added pyruvic acid or a derivative and found that the corresponding amino acid was formed. In this way they demonstrated the formation of tyrosine, alanine, phenylalanine and probably leucine. The first two amino acids were present in their natural optically active form. They and subsequently Fellner [1911] also showed with a glycogen-rich liver that if a small amount of ammonium chloride be added to the perfusing fluid there was a comparatively free synthesis of alanine. Alanine was also formed in

small amount even in the glycogen-poor liver if ammonium lactate were added to the perfusing fluid, although the perfusion of the glycogen-poor liver with ammonium chloride gave a negative result.

It is interesting to note that Kondo [1911] demonstrated that the synthetic power of the liver was not confined to the synthesis of amino acids which are normal constituents of the body: he was able to obtain *α*-amino *n.*-butyric and *α*-amino *n.*-caproic acids by perfusing the liver with the appropriate keto acids.

It may be taken as practically proved that carbohydrate in some form or other is absolutely essential for the synthesis of protein within the tissues.

The Influence of Non-nitrogenous Substances on the Rate of the Protein Breakdown.

(*a*) *During Inanition.*

The question may be considered from two points of view, the influence of carbohydrate and fat stores in the tissues on the rate of breakdown in inanition and the influence of these substances when fed to an animal on a protein-free or protein-low diet.

In the first instance the condition in starvation will be briefly considered. There is perfectly clear evidence that in the first day or two of a fast the output of nitrogen is comparatively low due to the presence of the glycogen store. In spite, however, of the fact that the carbohydrate is very readily used up it has been abundantly proved that practically throughout a long period of starvation the blood sugar remains very constant. Bloor [1914], Underhill and Baumann [1916, 2], have examined the fat content of blood in fasting and have found that on the whole it too remains wonderfully constant. Underhill and Baumann observed that when a condition of hypoglycæmia was induced by means of hydrazine there was a tendency for the blood fat to rise.

The fact must not be lost sight of that in starvation the amount of nitrogen liberated—protein utilized—ultimately depends on the supply of fat in the tissues. So long as there is a sufficiency of fat present in the organism the nitrogen output slowly falls, but as soon as the fat store is all utilized there is a rise in the output of nitrogen. This fact has been clearly demonstrated by a large number of workers. C. Voit [1881], Schöndorff [1897], Rubner [1881], E. Voit [1901], and others. Voit [1881] was the first to show that the administration of fat to a starving animal tended rather to increase the extent of

protein metabolism than to diminish it. It has, however, been clearly demonstrated that an animal with large stores of fat will resist starvation better than one which is thin. Schulz and his pupils [1906], on the other hand, hold that the premortal rise in the output of nitrogen is not due to the lack of fat or other nitrogen-free food material, but to a general breakdown of the cells themselves as the result of injury due to the hunger. E. Voit denies this. He holds that death in starvation arises not from the death of the total cell mass of the body, but from nutritional disturbances of essential organs. Reicher [1909] also is inclined to accept this view. This worker was never able to detect any evidence of necrosis. He quotes some work of Loeb in which it was shown that cell disintegration first took place after the removal of certain important lipoids (compare the work of Glikin and Stepp, p. 88). Schulz maintains that the premortal rise takes place even if the animal be given fat or carbohydrate in sufficient amount to prevent loss of fat from the tissues. Kaufmann [1901], who repeated some of this work, found that rabbits in a fasting condition died in three to four days with a persistently high output of nitrogen even if they were given oil. On the other hand, when cane sugar was introduced the results obtained were much more satisfactory; he was able to keep the animals alive for even nineteen days with a constant decrease in the amount of nitrogen excreted. Heilner [1910] also found that the administration of fat to the starving animal led to an increase in the output of nitrogen, whereas the giving of cane sugar [1911] lowered the metabolism of protein; he did not, however, believe that this was evidence of protein sparing. Wimmer [1912] in a very careful study of starvation showed very definitely that carbohydrate led to a very considerable saving of protein. It was immaterial whether the carbohydrate was given as glucose or starch. Yoshikawa [1911] found that there was no output of amino acids in the urine of starving animals until just a day or two before exitus. If, however, the fasting animals were depleted of their carbohydrate by means of phlorhizin there was an immediate rise in the output of amino acids even on the second day of the fast, and Oehme [1914] noted that if he injected a starving animal intravenously with erepton there was a rise in the ammonia output in the urine. When the injection was made into animals fed on carbohydrate the rise did not occur.

(*b*) *As Sparers of Protein when Fed.*

This phase of the question is one of particular interest as obviously it is very intimately related to the size of the protein minimum which

is dependent on the nature and the amount of non-nitrogenous material fed. Perhaps the most striking effect of the ingestion of carbohydrate or fat is at the end of a period of fasting, i.e. when the tissue material is not only covering the wear and tear quota but also the dynamic one. Thus, in the case of Beauté, at the conclusion of the fasting period the subject was given a diet consisting of cream and starch with the result that, within three days, the output of nitrogen had fallen to one-third of the output on the last (fourteenth) day of the fast. Rubner has also observed the same effect in the case of dogs. Unfortunately Benedict was unable to get any observations of value on this point with his subject L.

A very large body of literature dealing in some instances directly but more often indirectly with the problem of the influence of the non-protein food on the nitrogen output is available. A fair number of experiments have been carried out to determine the effects of carbohydrate and of fat on the protein catabolism. Thus Kayser [1893] found that he could not replace the carbohydrate in a diet with an isodynamic amount of fat without a rise in the output of nitrogen, and Tallquist [1902] and Hellesen [1903] both found that with a carbohydrate-rich, fat-poor diet there was a positive nitrogen balance, and with a fat-rich, carbohydrate-poor diet there was a tendency towards a negative nitrogen balance. The whole question assumed a new importance with the excellent work of Landergren [1903] who found that there was a very real difference in the power of carbohydrate and fat to spare protein. This result is very clearly shown in the accompanying table. It is evident that, when the subject is placed on an exclusively carbohydrate diet, there is a steady fall in the output of nitrogen and that when the diet is changed to one exclusively of fat there is just as steady and progressive a rise. It is much to be regretted that, owing to the nature of the diet and the results it produces, it is practically impossible to continue the experiment with fat over a longer period than three days.

Carbohydrate.						Fat.					
Landergren.		Cathcart.				Landergren.		Cathcart.			
		I.		II.				I.		II.	
1	8·91	1	6·40		Only two days on this diet.	5	4·28	5	4·83	3	5·25
2	5·15	2	4·77			6	8·86	6	8·13	4	9·01
3	4·30	3	4·79	1	8·12	7	9·64			5	13·30
4	3·76	4	4·39	2	6·65						

As regards the effects of the non-protein diet on the protein metabolism, Landergren maintained that it all depended on the various purposes served by the nitrogenous material in the organism. He held, that in starvation, for instance, there were three functions undertaken by the protein: (1) to supply the nitrogen required to cover wear and tear, (2) to keep up the carbohydrate supply in the tissues and fluids, and (3) to serve as complementary protein used for dynamic purposes. He believed that the first could, naturally, only be covered by the ingestion of protein, the second could be spared by a sufficiency of carbohydrate but not by fat, and the third, the complementary protein could be replaced by any food-stuff. Cathcart [1909], who fully confirmed these results, by another line of attack, was able to show definitely that the two food-stuffs exerted different actions on the protein catabolism. In a further series of experiments, not yet published, in which the subject was fed either on fat or carbohydrate or mixtures of these substances, he found that as the carbohydrate content of the diet was raised not only was there a very definite influence on the creatine output but there was also a steady rise in the output of uric acid. Curiously enough in each of these experiments, when only olive oil was given, the total nitrogen output was definitely higher on the second than on the third day. Graham and Poulton [1913] and Umeda [1915] have also noted the effect of giving fat and carbohydrate on the purine output (p. 122).

Rosenstern [1911] working with children, found that, if he replaced some of the sugar in a comparatively carbohydrate-poor diet by an isodynamic amount of fat, there was an immediate drop in the weight of the infants; the weight again increased immediately sugar was restored to the diet. He admits that a retention of water, brought about by the carbohydrate ingestion, may play some part, but it cannot account for all the changes observed. Zeller [1914] has also investigated the problem. He fed both dogs and man with varying quantities of carbohydrate and fat. As the result of these experiments he came to the conclusion that from 70 to 90 per cent. of the carbohydrate in a diet could be replaced by an isodynamic amount of fat without the nitrogen minimum, reached by exclusive carbohydrate feeding, being materially effected. He maintained that for the complete combustion of fat there must be at least one part of carbohydrate to four parts of fat. It may be mentioned in this connexion that Lang [1915] found in his observations on the production of acidosis that acetone appeared in marked amount when the carbohydrate fat ratio was one to two. Zeller further found that, when the diet

consisted wholly of fat, a very definite rise in the output of nitrogen took place due, presumably, to increased protein breakdown. He noted that, on the 100 per cent. carbohydrate diet, the urea percentage of the total nitrogen fell to a low figure (below 40 per cent.), and that, as previous observers had noted, the nature of the diet had a very definite influence on the amount of purine, particularly uric acid excreted. The output of neutral sulphur was increased in the case of the fat rich diets. Umeda [1916] attacked the problem in another fashion by superimposing caseinogen or gelatin on three different diets: I. Carbohydrate rich, fat poor; II. Intermediate; III. Fat rich, carbohydrate poor. He found, as the following figures clearly demonstrate, that the retention of nitrogen was most marked in both instances in the case of the carbohydrate rich diet.

	I.	II.	III.
Caseinogen, Retention of N.	35 per cent.	28·6 per cent.	8·5 per cent of added N.
Gelatine " "	20·7 "	—	9·7 " " "

He also found that the addition of meat extract to gelatin did not increase the amount of nitrogen retained. This observation is of interest in the light of the conclusion of Thomas [1909] (see p. 83).

Ringer [1912] noted that the sparing action of carbohydrate could even be demonstrated in a dog rendered glycosuric by means of phloridzin, in spite of the fact that the sugar given was apparently excreted forthwith in the urine unchanged. He strongly supported the attitude taken up by Landergren with regard to the types of protein metabolism. Kocher [1916] has also found that lactic acid which, in all probability, is a normal degradation product in the course of carbohydrate metabolism, can act as an efficient sparer of protein, although, curiously enough, pyruvic acid did not show the same capacity.

On the other hand, both Maignon and Bartmann have definitely stated that fats have also the power of sparing proteins in a marked degree. Maignon [1912], who experimented with young dogs and rats, found that they died if they were fed on an exclusively protein diet due, in his opinion, to the fact that exhaustion of their fat depôts occurred. He found that an addition of fat, which was not replaceable by carbohydrate, was necessary in order to bring about satisfactory nutrition. Bartmann [1912] also came to the conclusion, although he frankly admitted that the giving of fat frequently seemed to increase the catabolism of protein, that, provided care be taken, fats possess a protein sparing action. In those cases where he obtained, apparently, an increased protein catabolism, he maintained that the increased metabolism of the protein was not directly due to the fat

but to irritation of the intestine caused by the fat which led to an increased loss of nitrogen by way of the fæces.

Even if the protein be fed with the fat, as in the experiments of Voit and Korkunoff [1895], the fall in protein catabolism, although it reaches a level well below that for the protein alone, is not so marked as in the case of protein and carbohydrate. Thomas [1910] observed a similar reduction with fat, and Rubner [1903] has shown that, when increasing quantities of fat are added to a diet in which the nitrogen and carbohydrate content are kept approximately constant, there is an increasing and definite addition of protein to the body.

It is impossible in the state of our present knowledge to offer an adequate explanation for the increase in protein catabolism or, perhaps more accurately, for the increase in the output of nitrogen in the urine so commonly observed after the giving of a fat-rich or exclusively fat diet. It may be that the extreme degree of acidosis produced by such food is the sole causal factor. As L. J. Henderson and many others have shown, the whole effort of the organism is towards the maintenance of the alkaline reaction of the tissue fluids, hence it is possible that any condition, such as exclusive fat feeding, which leads to an excessive and rapid production of acid products might induce an increase in protein catabolism. That the reaction may play some part also emerges from the interesting work of McCollum and Hoagland [1913] who found in their experiments on pigs that the nature of the salt mixture fed with the fat ration materially altered the course of the nitrogen output in the urine. The work of Underhill and Baumann [1916, 1] shows quite conclusively, however, that acidosis cannot account for all the results.

Falta, Grote and Staehelin [1907] contend that the increased nitrogen output is due to the fact that the demand for carbohydrate is so urgent that protein is catabolized in order to obtain it. Landergren was very much of the same opinion. He considered that for some as yet unknown reason carbohydrate was in constant demand by the tissues. Under physiological conditions, at least, this demand could not be met by fat, and that therefore, when the supply of carbohydrate from without was lacking, protein tissue was broken down to supply it, with the result that there was a rise in the output of nitrogen in the urine. It is quite possible that the unknown demand postulated by Landergren is the necessity of a constant supply of carbohydrate in the tissue fluids for various intracellular activities, ultimately probably for protein synthesis.

The fact remains that there are no grounds for maintaining the fiction of speaking about the possibility of replacing fat and carbohydrate in isodynamic amounts in the diet over the whole range of replacement. Rubner [1919] himself states that it is absurd to speak of the complete removal of fat from the diet. It is to be regretted that the term isodynamic replacement is so freely used, as it infers that the whole basis of the exchange is founded on mutual replacements for energy requirements, whereas modern work is daily showing more and more clearly, that the purposes served by the non-nitrogenous food-stuffs, so far as the tissues in contradistinction to the whole organism are concerned are not confined solely, nor indeed probably primarily to the supply of energy. As Chauveau [1907], Gigon [1911], and others have stated, with varying degrees of emphasis, it is quite impossible to view all the functions of food-stuffs as being more or less identical, although the end products of their metabolism, apart from the actual nitrogen, are very closely akin. We cannot then logically refer to the replacements of the various food substances in terms of energy, although it is very obvious, from the work that has been done, that there is some relation in the amounts of the various substances which can replace one another in a diet. It would therefore be preferable to refer to these replacement values rather in terms of their "sparing" value than their dynamic value. Such a term as isotamieutic (ταμιευω = to husband or to spare) would be both more expressive and more physiological than isodynamic.

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